

USACEHR TECHNICAL REPORT 0201

**OPTIONS FOR DEVELOPMENT OF ENVIRONMENTAL SENTINEL BIOMONITOR
SYSTEMS FOR REAL-TIME DETECTION OF TOXIC CHEMICALS
IN RESPONSE TO US MILITARY NEEDS**

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13. ABSTRACT (Maximum 200 words) This report summarizes the substance of a workshop on environmental sentinel biomonitor systems (ESB) held 26-28 Sept 2001 at the US Army Center for Environmental Health Research. The focus of the workshop was on development and field use of ESB systems as real-time early warning sensors for the presence of toxic industrial chemicals and toxic industrial materials (TICs and TIMs) which might present health hazards to exposed personnel. The scope of the workshop was limited to human health considerations and issues, and a number of military scenarios were discussed.			
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LIST OF ACRONYMS

ACTD	Advanced Concept Technology Demonstration
BWA	Biological Warfare Agent
CONUS	Continental US
CWA	Chemical Warfare Agent
DoD	Department of Defense
ESB	Environmental Sentinel Biomonitor
ETV	Environmental Technology Verification
FHP	Force Health Protection
JORD	Joint Operational Requirement Document
ICCVAM	Interagency Coordinating Committee for the Validation of Alternative Methods
MASINT	Measure and Signature Intelligence
MNS	Mission Need Statement
NBC	Nuclear, Biological and Chemical
OCONUS	Outside the Continental US
OEH	Occupational and Environmental Health
ORM	Operational Risk Management
R&D	Research and Development
TIC	Toxic Industrial Chemical
TIM	Toxic Industrial Material
US	United States
USACEHR	United States Army Center for Environmental Health Research

INTRODUCTION

This report summarizes the key observations and recommendations from the Environmental Sentinel Biomonitor (ESB) System Workshop sponsored by the US Army Center for Environmental Health Research (USACEHR). The Workshop was held 09/26-28/01 in Frederick, MD.

For this project, ESB systems are defined as:

- Biologically-based systems (in vivo, in vitro, synthetic) and chemical sensors placed in the environment (in situ), either mobile or stationary, that can sense a biologically-significant event and provide relevant real-time data for use in risk assessment, mitigation, and/or management.
- Real-time data are not limited to data available instantaneously to the user/decision maker. Data available within the time frame required by the military decision maker are considered real-time for this project. This is envisioned to typically be less than one hour.

The overriding questions the project attempted to answer are:

1. Can ESB systems be useful to the military's Force Health Protection (FHP) program?
2. If they can be useful, how can ESB systems contribute most to the military's FHP program? What types of data and information are needed? Under what military conditions/scenarios should ESB systems be expected to operate?
3. Is research needed to better answer the two questions above? And if so, what scientific and technical challenges must be overcome and what data and information must be generated?

The focus was on the scientific, not the engineering, aspects of integrated ESB systems, achievable within five years (referred to as near-term) and those achievable within five to 10 years (referred to as far-term), with the potential to ultimately provide continuous, real-time monitoring of single known and unknown Toxic Industrial Chemicals/Toxic Industrial Materials (TICs/TIMs) and their complex mixtures under anticipated military field conditions and operational scenarios integrating both biological effects and chemical measurements and possibly triggering supplemental efforts (e.g. sample collection and/or additional analyses).

The Workshop Agenda, Attendees' List and Workshop Charge are in Appendices A, B and C, respectively. The White Paper (Appendix D) was peer reviewed and copies distributed to all persons invited to the Workshop for comment. It has not been formally staffed.

APPROACH

The objectives of the project included:

- Determining if ESB systems have the potential to satisfy certain materiel portions of existing and/or possible future Mission Need Statements (MNSs).
- Proposing critical system performance capabilities for ESB systems. These capabilities could ultimately be incorporated into a future Joint Operational Requirement Document (JORD).
- Evaluating potential ESB system alternatives/options/configurations conceptually taking into consideration:
 - ◆ The readiness level of the technologies and technology barriers.
 - ◆ Potential performance parameters and capabilities for military field use.
 - ◆ Applicability to military conditions envisioned by users during:
 - ▶ Major Theaters of War
 - ▶ Single small contingencies involving conflict
 - ▶ Sustainment and Support Operations
 - ▶ Terrorism incidents (at home and abroad)
 - ▶ Accidental chemical spills/releases affecting military personnel's health at continental US (CONUS) and outside the continental US (OCONUS) locations
- Assessing the potential of ESB systems to assist preventive medicine personnel at all command levels assigned the task of providing health risk assessments to the commander based on Occupational and Environmental Health (OEH) surveillance.

Figure 2-1 depicts the principal tasks of the project and identifies the documents prepared during the project.

The overall approach to the project is depicted in Figure 2. Blocks 1, 2, 3 and 4 in Figure 2 were introduced in the Concept Paper and expanded upon in the White Paper. The Workshop provided an opportunity for representative researchers and potential military "users" of ESB systems to engage in a direct exchange of views and information extending over three days. The terrorism occurrences of 09/11/01 forced a number of military "users" to cancel their plans for attending the Workshop. Although greater participation from military "users" may have contributed additional perspectives (e.g., Special Forces, Quartermaster School, Theater Army Medical Laboratory, Chemical School, Navy Environmental and Preventive Medicine Unit), "user" input was provided by the attendees who had previous deployment experience and/or are actively engaged in the FHP of the military.

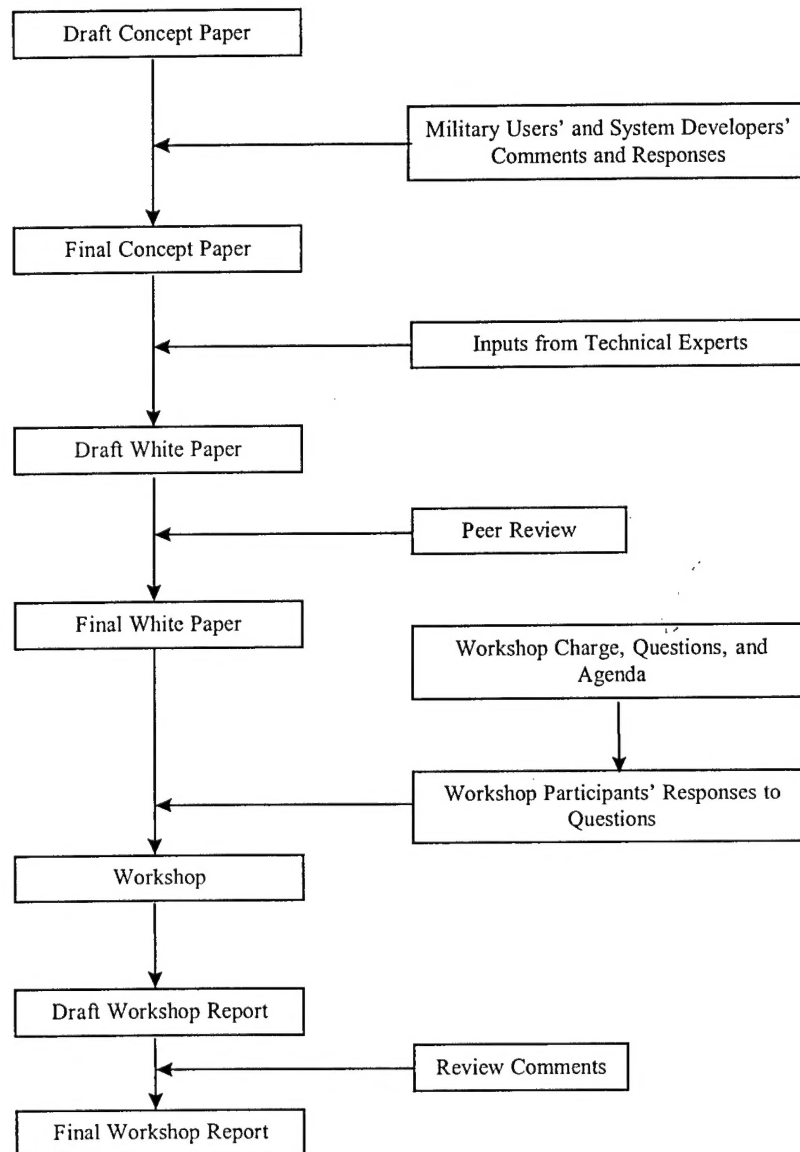


FIGURE 1 - PROJECT'S PRINCIPAL TASKS AND DOCUMENTS

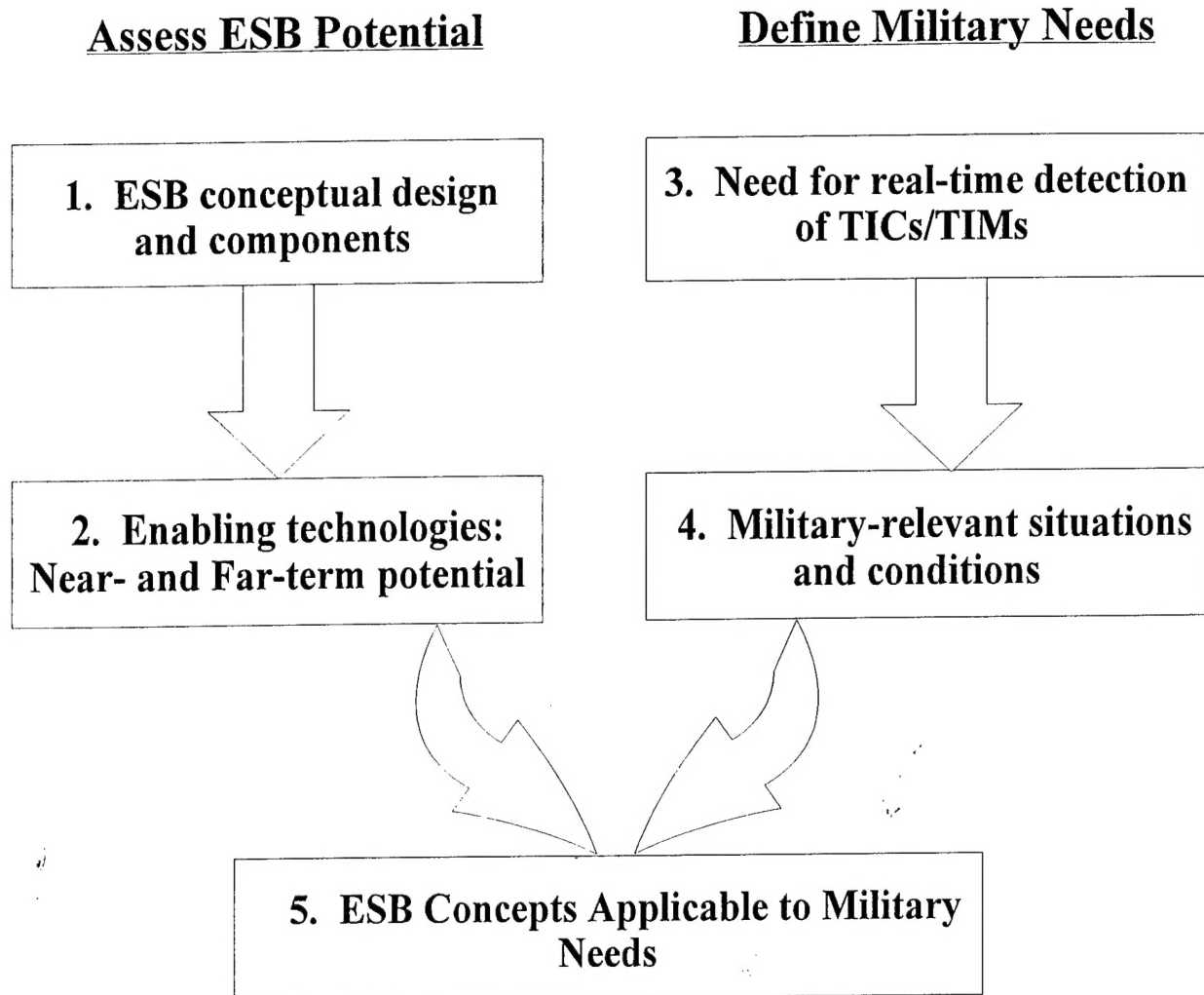


FIGURE 2 - OVERALL APPROACH

OBSERVATIONS AND RECOMMENDATIONS

Although there was no attempt to achieve consensus among the Workshop participants, a number of observations and recommendations can be drawn from the Workshop. The order in which the following observations and recommendations are presented below does not imply their perceived importance or priority. Certain observations do not have an accompanying recommendation. Observations and recommendations stated during the closing session by a participant or group leader start on page 5. A summary of the observations from the three breakout sessions that were not restated during the closing session were prepared with the assistance of the breakout session group leaders, Drs. Robert Cartledge and Hank Gardner. These observations start on page 8.

Closing Session Observations and Recommendations of Workshop Participants

The following observations and recommendations were made:

1. It is better to try a 20 percent solution today than delay and attempt to develop and field the perfect system. Development should take an iterative approach and target opportunities, such as acute health effects, which appear to be achievable in the near-term. ESB systems could be considered for demonstration as part of an Advanced Concept Technology Demonstration (ACTD) or some other military field demonstration of ESBs. ACTDs are conducted in a military operational environment with active user and developer participation. User evaluation in the field is an approach to getting support from field commanders. Rapid prototyping is preferred. A modular/systems approach is attractive because new technologies can be inserted as they become available to demonstrate their value. The alternative is to get the need documented in a MNS and performance capabilities spelled out in an approved JORD. This approach is likely to take considerable time and does not provide as much linkage to field users. A presentation on ESB systems and a proposal for their evaluation should be made at the earliest opportunity to the Joint Environmental Surveillance Working Group. Presentations to other military groups (e.g. Joint Water Resources Management Action Group) and military organizations (e.g., Engineer, Medical, Chemical and Quartermaster Schools) should be pursued.
2. Environmental exposures to TICs/TIMs have not been considered nearly as great a health threat to the deployed warfighter as have potential exposures to Chemical Warfare Agents/Biological Warfare Agents (CWAs/BWAs).
3. Since there are so many human health endpoints, thousands of TICs and even greater numbers of potential chemical mixtures to which individuals may be exposed, there is a need to focus efforts and identify valid models for specific endpoints. Selecting a small number of TICs (five to six) to use for evaluating and comparing various ESBs in various laboratories may be a useful next step. Evaluation of ESBs at a single central location is another possibility. A single location evaluation with a set test protocol is likely to provide more useful data than if tests are conducted in numerous labs and/or with several test protocols.

4. ESB systems for use by the military need to be "user-friendly." They need to provide useful data within the military's existing decision-making framework.
5. There is an increased awareness and concern by the military of delayed and chronic adverse health effects from possible low-level exposures to CWAs and other stressors (chemical and physical). Although Nuclear, Biological and Chemical (NBC) defense and Occupational and Environmental Health (OEH) responsibilities are not integrated within DoD, the recent military guidance for commanders making Operational Risk Management (ORM) decisions in the field to take OEH hazards and exposures into account requires that information on both NBC and OEH hazards and exposures be available to commanders. Consideration should be given to integration of "skin out" (e.g., ESB and other environmental health assessment information) and "skin in" (e.g., Warfighter Personal Status Monitor) systems for a full spectrum environmental health threat surveillance and assessment capability. In addition to the technological, data fusion/integration, and risk integration and communication challenges, the interdisciplinary, inter-command and inter-service doctrine and policy challenges will potentially be formidable. These integration challenges should not dissuade USACEHR from engaging these issues, however. Full spectrum Environmental Health Protection for DoD personnel and new dual-use technologies for homeland defense are at the heart of many policy and doctrine statements from a variety of sources in DoD. They are also central to outside reports (e.g., National Academy of Sciences and Institute of Medicine), providing advice to the DoD on OEH and NBC hazards, exposures and risks.
6. Evaluation mechanisms, such as the Environmental Protection Agency's Environmental Technology Verification (ETV) and the Interagency Coordinating Committee for the Validation of Alternative Methods (ICCVAM), were mentioned. Neither of these mechanisms is ideally suited for evaluation of ESB systems for military field use, but an ICCVAM-type process may be useful in evaluating some ESB methods because it would provide a framework for evaluating the suitability of non-mammalian toxicity testing approaches.
7. ESB systems are effects-based monitoring that may also include chemical-based monitoring. Information from ESB systems will be useful to the military's FHP only if there is an accompanying paradigm shift from the common chemical-by-chemical approach to human health risk assessment for exposures to chemicals. Given that the use of ESBs will involve a paradigm shift in system acquisition and deployment, a different approach to the management of the development process might involve the following:
 - Define a straight-forward military application to demonstrate the ESB concept.
 - Identify a DoD partner who will champion the ESB concept (e.g., intelligence).
 - Develop a simple prototype to demonstrate ESB utility.
 - Conduct iterative demonstrations with the DoD partner to refine the concept.
 - Test the refined ESB concept in an operational environment, and if successful, leave test units with the users for further evaluation and developmental feedback.

- Propose the ESB concept as an ACTD to gain military user visibility.
- Repeat the above process with other ESB technologies using more complex applications.
- Use rapid prototype development to refine ESB features and capabilities.

Because of the novel nature of the ESB technologies, it will be necessary to build and demonstrate prototypes in user environments, so users can see their utility in operation. If successful, the word will spread at the user level, both within the military and commercial sectors. Classic military science and technology efforts with technology transitions based on approved acquisition programs (defined threats and validated requirements) are unlikely since ESBs will be viewed as having a low probability of success.

8. ESB systems are likely to provide information that will complement not replace the need for chemical sensors. ESB system response could be used to trigger on-line chemical analyses to identify and quantify the chemicals causing the response. Certain on-line chemical analyses could be used to trigger activation of the ESB system. Using integrated information from ESB systems as well as chemical sensors will improve the chance of success and user acceptance. The vast majority of ESB-based research have been directed to proof-of-concept of individual technologies. Efforts to combine multiple biomonitoring technologies with complimentary endpoints in systems and networks have been limited. ESB systems will not replace the need to detect, identify and quantify individual CWAs and TICs/TIMs in air, water, soil and food and on surfaces, clothing and skin. ESB systems, however, have the potential to provide information not otherwise available, a toxicological response.
9. ESB systems could be used to determine the "all clear/safe" condition for DoD personnel after an incident or be used as a continuous sentinel for "first alert."
10. Noteworthy is the potential for ESB systems to provide data not otherwise available, such as toxicological response to chemical mixtures and response to intermittent exposures. However, data generated by ESB technologies may be and likely will be coupled with other non-ESB technology generated data for use in risk assessment and ORM decision making.
11. Developing and presenting ESB system response in some form of "equivalences" to a human health standard or other established benchmark of human toxicity appears to be a workable and useful concept.
12. Critical scientific, technical, testing, and engineering issues remain for the ESB research communities to address. It is recommended that the following be considered for technology investment and insertion into an ESB developmental roadmap:
 - ESB hardening and miniaturization for field deployment applications.
 - Correlation of ESB data to various human health risks.
 - Controlling and interpreting biological variability.
 - Storage of biological tissues for use during deployment.
 - Development of an ESB system testbed.
 - Data fusion of ESB information across ESB system networks.

- Insertion of ESB system output into the military's ORM process for OEH hazards and exposures.
 - Benchmarking ESB technologies against current systems to define the value of ESB system concepts.
 - Value of information about abnormal cellular activity as compared to structural identification.
13. Military working animals, dogs and marine mammals are environmental sentinels, and they can provide a useful source of data for human health risk assessment. The presence/absence and health status of indigenous animals in a deployment area can also yield information about TIC exposures.

Breakout Session Observations and Recommendations

The first breakout session had three breakout groups formed from the Workshop participants. Because there were fewer attendees on the second day of the Workshop, the three original groups were consolidated into two groups for the final two breakout sessions.

Summary of Breakout Session No. 1

During this session the groups were asked to develop a set of military scenarios for ESB systems and decide upon important operational capabilities for ESB systems in these scenarios.

The groups acknowledged that it is impossible to incorporate all combinations of all conditions important to ESB system performance requirements in a few scenarios. However, the breakout groups did converge in recognizing that the military mission, mission duration, the location of the military operation, the force size and type, and the TIC/TIM and/or CWA/BWA threat potential formed significantly different system needs and operational capabilities.

One breakout group developed the following three scenarios:

Scenario I

Mission: Temporary "bed-down" of US military forces.

Duration: 8 weeks.

Force Size: Approximately 1,000 personnel (all active duty military).

Hazard: TICs.

Location: Near city that is unfriendly to US and contains industrial plants.

ESB Operational Capabilities: Continuous operations, molecular-based technologies, transportable, few consumables, complex water input, real-time air monitoring with stand-off detection, real-time analysis, data archives for post-mission analysis.

Scenario II

Mission: Long-term peacekeeping duties by US forces including combat service support operations.

Duration: 1 year.

Force Size: Approximately 10,000 personnel (military, civilian, contractor, elected officials).

Hazard: TICs and NBC (low probability); US hazardous materials.

Location: Remote rural.

ESB Operational Capabilities: Fixed, long stand-off sensing, networked, specialized technicians, simple water monitoring, pre-deployment site choice, real-time analysis less critical, effects of chronic exposures a concern.

Scenario III

Mission: Rescue of US personnel from US embassy.

Duration: Less than 24 hours.

Force Size: Approximately 100 personnel (military, evacuees).

Hazard: Smoke and particles from fires.

Location: Remote rural.

ESB Operational Capabilities: Small, mobile, sample collection (air), sensors with satellite uplinks air dropped prior to deployment (Measure and Signature Intelligence (MASINT) applications), unmanned aerial vehicles with an ESB system in the nose cone and post-deployment analysis.

The other two breakout groups focused more on the types of information personnel at different organizational levels and with different responsibilities would want from ESB systems. It became obvious that the exposed individual, field preventive medicine technician, unit surgeon and preventive medicine officers at various command levels, field medical laboratory personnel, etc. will want different information and want the information in different forms.

Summary of Breakout Session No. 2

The Work Groups were asked during the second breakout session to discuss key technology components of ESB systems as applied to the scenarios developed during the first breakout session. One breakout group approached the key technology components based on the scenarios developed during the first session. Key technologies identified by this group are as follows:

- Scenario I: Water input media, gas chromatography, mass spectrometer, engineered cells, bioluminescent probes, compressed gas, "freeze dried" embryos (just add water) as source for cells, extended shelf-life cells, self-calibration systems, decision-making algorithms for health risk assessment.
- Scenario II: Input processors (air, windblown contaminants, soil and TICs), concentrators, filters (< 10 microns), dry-to-wet converters, carrier molecules, semipermeable membranes, nanotechnologies.
- Scenario III: Real-time alarms (auditory, visual, tactile), absorbents, air concentrator, filters.

The second breakout group developed a matrix comparing the various ESB system technologies/components on a number of parameters. The results are depicted in Table 1.

TABLE 1 - COMPARISON OF VARIOUS ESB SYSTEM TECHNOLOGIES/COMPONENTS

Technology/System Component	Speed of Response	Specificity	Sensitivity	Agent Range	Survivability	Security	Support Requirements	Quantitative Response
Whole Organisms	1	3	3	1	3	3	3	2
Tissue/Organ Based Biosensors	2	2	2	1	3	1	3	1
Cell-Based Biosensors	2	2	1	1	3	1	3	1
Sub-Cellular Biosensors	3	1	1	1	2	1	3	3
Biochemical Measures	2	1	1	2	1	1	2	1
On-Line Analytical Chemical Sensors	1	1	1	2	1	2	1	1
1 = Favorable Performance 2 = Less Favorable 3 = Least Favorable								

In Table 1 the designations "1," "2," and "3" were assigned by the group members indicating their relative capability on each of the eight parameters heading the columns of the matrix. The designation of "1" indicated favorable performance, "2" less favorable and "3" lower performance than "2". Where a "1", a "2" or a "3" is assigned to more than one technology, the group considered those technologies about equal in their capability or potential for that parameter. What should be taken into account are the following:

- The process of making the evaluations was informal. The Work Group members had discussions on each of the eight parameters.
- The comparisons were based on the Work Group members' personal assessments based on their knowledge of the present state of each technology.
- The table does not take into account that only ESB systems can provide a measure of toxicity.
- Definitions for the eight parameters were not spelled out and agreed upon by the Work Group members. Therefore, the Work Group members made their evaluations based on their own definitions for the parameters.

- The potential for technology components to have improved performance on certain parameters may have been considered differently by the Work Group members.

What is evident from Table 3-1 is that on-line analytical chemical sensors currently have high favorability. This accounts for their wide use and acceptance as sensors, alarms and detectors. However, they cannot measure toxicity. The table also identifies technical challenge areas for the ESB technologies and suggests that a system comprised of several different type biosensors is an advisable and necessary direction for development.

Summary of Breakout Session No. 3

The final breakout session was devoted to discussing output applications of ESB system data to the military's OEH risk management. One group identified a range of developmental and deployment questions addressing risk management issues. These included the following:

- Who are the users of the ESB output?
- What are the categories for data analyses and interpretation?
- Who performs the analyses, interpretation, and risk communication?
- How will decision makers use the information?
- How quickly must the risk assessment be determined?
- What health risk alarms are needed (e.g., acute alarm requiring immediate action)?

Insertion into a military risk management system will require the following:

- Extensive reference databases.
- Probabilistic nomenclature using non-linear modeling.
- Lethality and subacute endpoints.
- Well-defined concept of operations that integrates into existing processes.

The group concluded that, in some cases, the challenges for inserting ESB information into deployment decision-making processes may be greater than development of the technologies themselves.

The second group used the approach that data applications drive actions. Applications that were identified included:

- Real-time response actions.
- Retrospective analysis of data and action.
- Additional monitoring decisions/actions.
- Identifying cause and mode of action of TIC/TIM exposure.

The group recognized that there will be hardware and software elements in all successful ESB systems. This is depicted in Figure 3-1 developed by the Work Group.

The expert system would generate output in "user friendly" formats for all of the targeted applications.

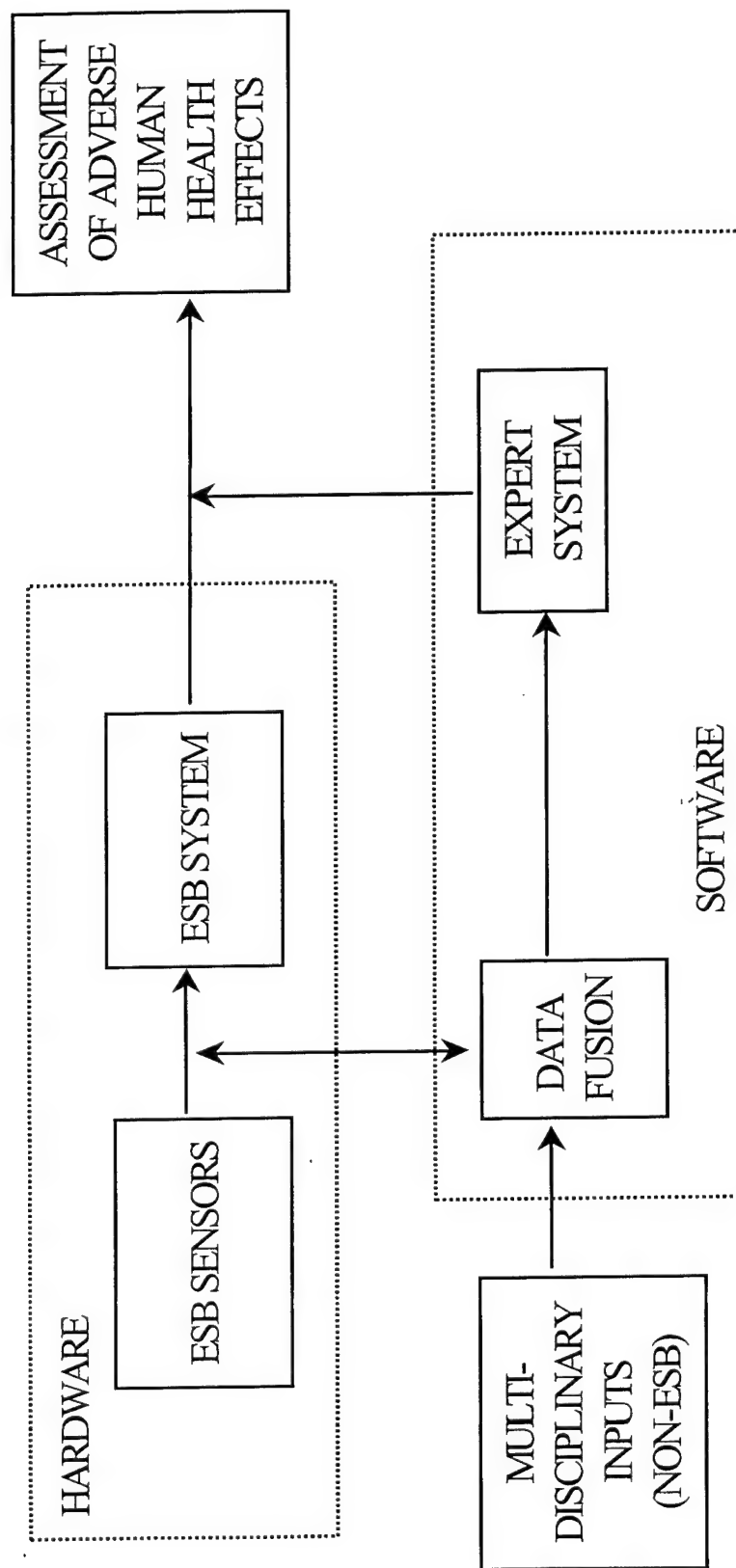


FIGURE 3 - CONCEPTUAL ESB SYSTEM

4.0

CONCLUSIONS

The conclusions listed in this section were developed from an assessment of the White Paper, supporting reference material and the Workshop presentations and discussions.

1. The Workshop, which is believed to be the first multi-day gathering of potential "user" representatives from each military service and researchers active in cell/tissue biosensors, on-line analytical chemistry, chemical biosensors, whole organism biosensors and gene microarrays, was successful in providing a forum for interaction among researchers and between potential "users" and researchers. The Workshop focused on utility of ESB systems in two major thrust areas: Military Operations and Occupational Health/Medical Surveillance. The participants concluded that ESBs have great potential to provide unique capabilities in both thrust areas. However, given that ESB systems represent non-traditional approaches to detection of health hazards, they will require atypical strategies to research, development, testing and deployment.
2. Military deployments are significantly different in many aspects from the civilian occupational/industrial environment. Military ESB system research must recognize these differences and account for them.
3. Deployable military units providing OEH surveillance and support currently rely heavily on commercial off-the-shelf equipment for analysis of TICs in all environmental media.
4. The need for more aggressive FHP pre, during and post deployment has been recognized by DoD and the services and put into military doctrine, policy and guidance.
5. The acceptance of ESB systems for military field use depends on:
 - The systems being as transparent as possible to the deployed force, and
 - The systems assisting field commanders facing potential TIC/TIM threats for which the military's existing capability is either unsatisfactory or unavailable.
6. The military has been directed to take into account OEH threats, including both immediate and delayed adverse health effects when using the ORM process for decision making. This applies even during deployments involving high-intensity combat.
7. The list of TICs in ITF-25 is generally accepted by the military as representing a good list of potential acute airborne TIC threats. The threat of airborne TICs/TIMs is considered a greater acute health risk than TICs/TIMs in field drinking water or in soil. When the ITF-40 report is completed, it will be a valuable information source for TIC/TIM deployment threats since it is considering water and soil media as well as air. Technical Guide 230, prepared by US Army Center for Health Promotion and Preventive Medicine, is the best compendium of guidance on a large number of individual TIC/TIM levels in air, water and soil for military medical personnel to use during deployments.

8. The military appears to be better equipped and trained to cope with the threat of troop exposures to CWAs than to incidents involving environmental exposures to TICs that are acutely toxic.
9. The military's Research and Development (R&D) program for CWA identification, detection and quantification is extensive. All of DoD's NBC defense research, development, test and evaluation and procurement funds are consolidated into defense-wide program elements. Almost all R&D in the US associated with sensors, monitors, detectors and alarms for CWAs is funded and managed by DoD. In contrast, the military's R&D efforts on ESB systems and related technologies are not centrally funded or managed. There is considerably more interest and activity among non-DoD government organizations and the private sector in funding ESB research than in research on CWA defense materiel.
10. Previously conducted and ongoing environmental biomonitor research has focused more on uses in natural water bodies and on wastewater treatment plant effluents than air, soil or water treated for potable use.
11. The usefulness of ESB system data for deployment FHP will be increased if:
 - The system response can be confidently and accurately related to the impact on the health of the deployed military personnel on a continuous real-time basis in a form useful to decision makers in the field.
 - The data enables decision makers to take action to avoid, minimize or eliminate the adverse health effects to the deployed personnel and not have an adverse impact on the military's mission.
 - Data records are established and maintained and integrated with data on personnel location, activity and health records.
12. Although the Workshop's focus was on ESB systems in support of deployed DoD personnel, it is apparent that ESB systems can also contribute to the protection of military personnel and civilians at permanent locations in the CONUS and OCONUS from accidental or deliberate releases of TICs.
13. Applications that address immediate acute health endpoints from temporary or short-term TIC/TIM exposures are considered the leading near-term opportunity for ESB systems.
14. Although it was not a primary topic at the Workshop, there was agreement that representative sampling, sample preparation and introduction are important technical challenges for ESB systems. Data fusion and networking and miniaturization will have to be addressed. However, they can be deferred until concepts are proven.
15. The variations in sensitivity of response and response variability of ESBs in comparison to humans represents a significant technical challenge. The more complex and variable the TIC exposure environment and ambient environment, the greater the challenge.

16. There is no civilian environmental regulatory requirement related to ESB systems for human health protection. Therefore, there are no standardized protocols to use to evaluate ESB systems.
17. The existing MNSs for hazards from industrial chemicals and for FHP against NBC and TIMs are documents ESB system developers should carefully review and consider in their programs because these documents provide insight to the users' needs.

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**APPENDIX A
WORKSHOP AGENDA
SEPTEMBER 26, 2001**

MORNING SESSION 0830 - 1230

- Welcome - COL Driggers
- Administrative Announcements - Dr. Reuter
- Self-Introductions of Participants
- Opening Remarks (The Charge, Workshop Organization) - Dr. Reuter
- Summary of Comments Received on the charge questions and White Paper - Dr. Reuter
- Current Military Field Capabilities for TIC Presented by Invited Service Representatives
 - ▶ Theater Area Medical Laboratory + USACHPPM Detachment - Mr. John Resta, USACHPPM
 - ▶ Air Force Capabilities & Deployed Environmental Surveillance System - Mr. Donald Lowe, Air Force Protection Battle Lab
- Health Risk Management for Deployed Personnel, Technical Guide (TG) 248 + TG 230 - Ms. Veronique Hauschild, USACHPPM
- The TIC/TIM Threat, International Task Force (ITF) - 40 Mr. John Resta, USACHPPM
- Ongoing CWA Detector. Alarm. Monitor & Sensor R&D Fielded System and their applicability to TICs - Dr. David Evans - ANSER

BREAKOUT SESSION 1 CHARGE

LUNCH

AFTERNOON SESSION 1300 - 1615

1300 - 1515

First Breakout Session^(a)

Topic: Develop a Set of Military Scenarios for ESB Systems and Decide Upon Important Operational Capabilities for ESB Systems for These Scenarios

1515 - 1615

Reports by Each Group + Facilitated General Discussion

BREAKOUT SESSION 2 CHARGE

^(a) For assignments to breakout groups see attachment.

WORKSHOP AGENDA - CONT'D
SEPTEMBER 27, 2001

MORNING SESSION

0830 - 1200

0830 - 1045

Second Breakout Session

Topic: Discuss Key Technology Components of ESB Systems
as Applied to the Scenarios Developed during the First
Breakout Session

1045 - 1200

Reports by Each Group + Facilitated Discussion

BREAKOUT SESSION 3 CHARGE

LUNCH

AFTERNOON SESSION

1300 - 1630

1300 - 1515

Third Breakout Session

Topic: Output Applications of ESB System Data to Military's
OEH Risk Management

1515 - 1630

Reports by Each Group + Facilitated Discussion

SEPTEMBER 28, 2001

MORNING SESSION

0830 - 1200

0830 - 1130

Open Discussion of Issues Raised During Breakout Sessions
(A panel made up of the Group Leads and moderated by the
Chair could lead the discussion and field issues/questions
raised by participants.)

1130 - 1200

Concluding Remarks + Summary of Results

- By Sponsor's Representatives
- Dr. Reuter

LUNCH

APPENDIX B

WORKSHOP LIST OF ATTENDEES

LTC Donald Archibald, US Army Medical Research and Materiel Command
Mr. Adam Becker, Marine Corps Systems Command
Dr. Wayne Bryden, The Johns Hopkins University, Applied Physics Lab
Dr. William Burrows, US Army Center for Environmental Health Research
Dr. Dennis Burton, University of Maryland
Dr. Robert Cartledge, Strategic Technology Decisions (Work Group Leader)
Dr. Michael Carvan III, University of Wisconsin, Great Lakes Water Institute
Dr. Eric Clegg, US Army Center for Environmental Health Research
Ms. Marianne Curry, US Army Center for Environmental Health Research
COL Donald Driggers, US Army Center for Environmental Health Research
Mr. David Evans, DTRA / ANSER
Dr. Hank Gardner, Colorado State University
Dr. Thomas Gargan II, US Army Center for Environmental Health Research/GEO-CENTERS
Ms. Veronique Hauschild, US Army Center for Health Promotion Preventative Medicine
Dr. Amanda Jenkins, US Army Research Laboratory
Dr. Paul Knechtges, US Army Center for Environmental Health Research
Dr. Jim Lazorchak, US Environmental Protection Agency
Dr. Donald Lowe, Air Force Protection Battlelab
Dr. Phil McFadden, Oregon State University
Dr. Charles Noss, Water Environment Research Foundation
Dr. Joseph Pancrazio, Naval Research Laboratory
Dr. Lars Piehler, US Army Research Laboratory
Dr. Roy Reuter, Life Systems, Inc.
CDR John Rossi III, Naval Health Research Center Detachment - Toxicology
CAPT Robb Rowley, US Air Force
Dr. Alan Rudolph, Defense Advanced Research Products Agency
Dr. George Schieferstein, US Army Medical Material Development Activity
Mr. Tom Shedd, US Army Center for Environmental Health Research
Dr. Bill van der Schalie, USACEHR / USEPA
Dr. Tom Waller, University of North Texas
Mr. Mark Widder, US Army Center for Environmental Health Research

APPENDIX C WORKSHOP CHARGE

The intent of the Workshop sponsor, the USACEHR, is to solicit input from all Workshop participants on the potential for ESB systems to be used by the US military as a component of the military's OEH risk management system to enhance the military's FHP.

Two important operational definitions for the Workshop are listed below:

- ESB Systems are biologically-based systems (*in vivo*, *in vitro*, synthetic) and chemical sensors placed in the environment (*in situ*), either mobile or stationary, that can sense a biologically-significant event and provide relevant real-time data for use in risk assessment, mitigation, and/or management.
- Real-time data are not limited to data available instantaneously to the user/decision maker. Data available within the time frame required by the military decision maker are considered real-time for this project. This is envisioned to typically be less than one hour.

The overriding questions for the Workshop are:

- Can ESB systems be useful to the military's FHP program? What types of data and information are needed?
- If they can be useful, how can ESB systems contribute most to the military's FHP activities? Under what military conditions/scenarios should ESB systems be expected to operate?
- Is research needed to better answer the two questions above? And if so, what scientific and technical challenges must be overcome and what data and information must be generated? Identifying who should perform the research is not a Workshop issue.

For this Workshop the focus is on:

- ESB applications for TICs/TIMs.
- Monitoring human health effects and/or exposures from water, air and soil.
- Technologies available now or within five years and within 5-10 years should be considered.
- The scientific, not the engineering, aspects of integrated-ESB systems for US military use. Enabling technologies for data dissemination, transmission, archiving, storage and retrieval, while important to ESB systems success, will not be addressed in detail.

This Workshop is limited to technologies potentially adaptable to military field operation. ESB applications solely for protection of environmental quality, not human health, are beyond the Workshop's scope.

APPENDIX D
WHITE PAPER

Development of Environmental Sentinel Biomonitor (ESB) Systems for Real-time Detection of Toxic
Chemicals in Response to US Military Needs

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INTRODUCTION

This White Paper is the second of a series of major planned documents to develop and define the US (United States) military's near- and far-term needs, operational capabilities and performance characteristics for ESB systems for real-time, continuous exposure and health effects monitoring of Toxic Industrial Chemicals and/or Toxic Industrial Materials (TICs and/or TIMs) to be used by the military for protection of Department of Defense (DoD) personnel before, during and after deployments and throughout their time in service. In addition, the project is to develop and evaluate options for ESB system design concepts for both the near- and far-term and explore their potential applicability to Chemical Warfare Agents (CWAs) and Biological Warfare Agents (BWAs).

Figure 1 shows the principal tasks of the project and the documents that will be generated.

The overall approach to this project is depicted in Figure 2. Blocks 1, 2, 3 and 4 were introduced in a completed Concept Paper. The first four blocks are being conducted in parallel and are expanded upon in this White Paper. Inputs from experts in environmental toxicology, online analytical chemistry, chemical biosensors, cell/tissue-based biosensors, gene microarrays and whole organism biosensors are included. The Workshop will examine and define the range of conceptual possibilities for Block 5, ESB Concepts Applicable to Military Needs.

Comments and responses to a series of questions in the Concept Paper were solicited from the following US Service and Defense organizations:

- 520th Theater Army Medical Laboratory (TAML)
- J4 Medical Readiness Division
- Marine Corps Systems Command
- Navy Environmental Health Center
- Navy Environmental & Preventive Medicine Unit (NEPU) No. 5 and 6
- US Army Center for Health Promotion and Preventive Medicine (USACHPPM)
- US Army Chemical School
- US Army Medical Center and School
- US Army Quartermaster School
- US Army Training and Doctrine Command
- US Central Command
- Army Research Office
- Defense Advanced Research Projects Agency
- Naval Research Laboratory (NRL)
- US Air Force Research Laboratory
- US Army Institute for Environmental Medicine
- US Army Medical Material Development Activity
- US Army Medical Research and Materiel Command, Research Area 4
- US Army Medical Research Institute for Chemical Defense
- US Army Research Laboratory (USARL)
- US Army Soldier, Biological and Chemical Command

The Concept Paper was also distributed to representatives at the following non-DoD organizations for comment:

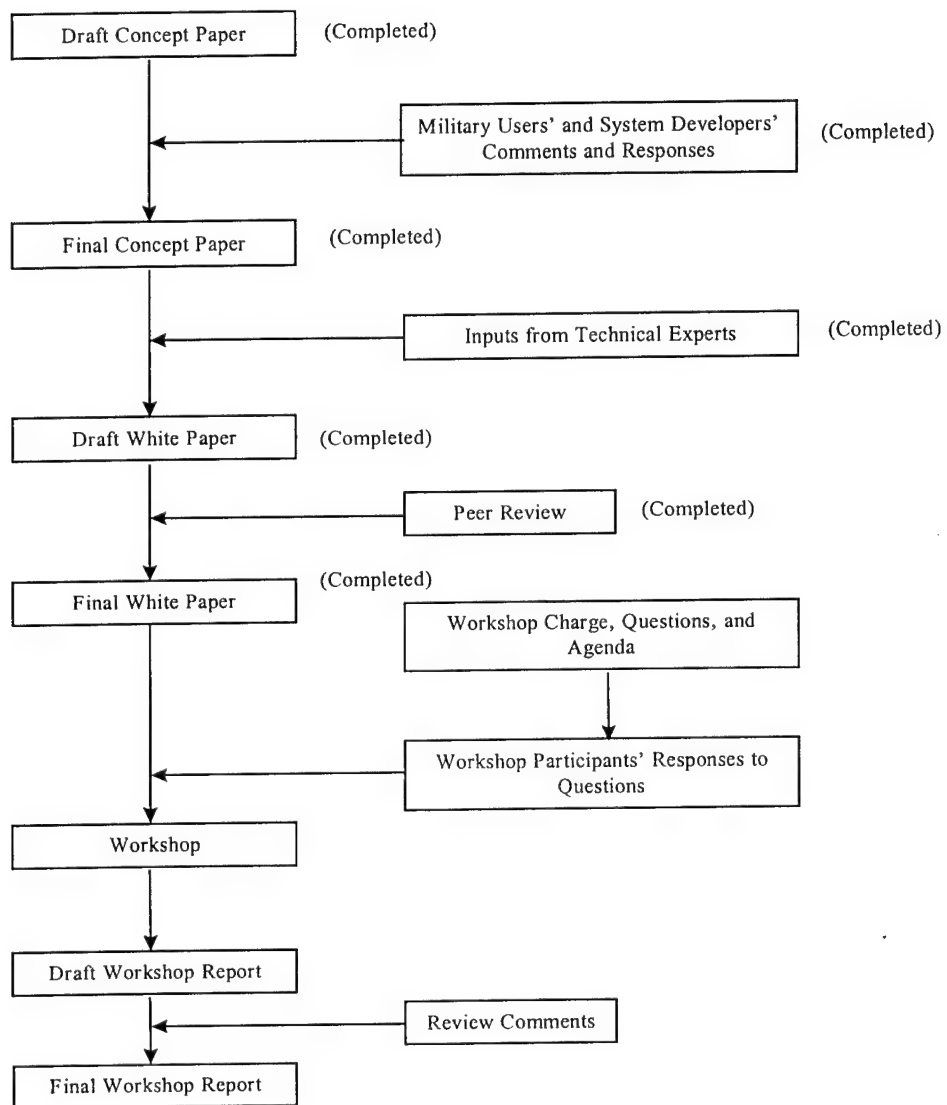


Figure 1. Principal Tasks and Documents

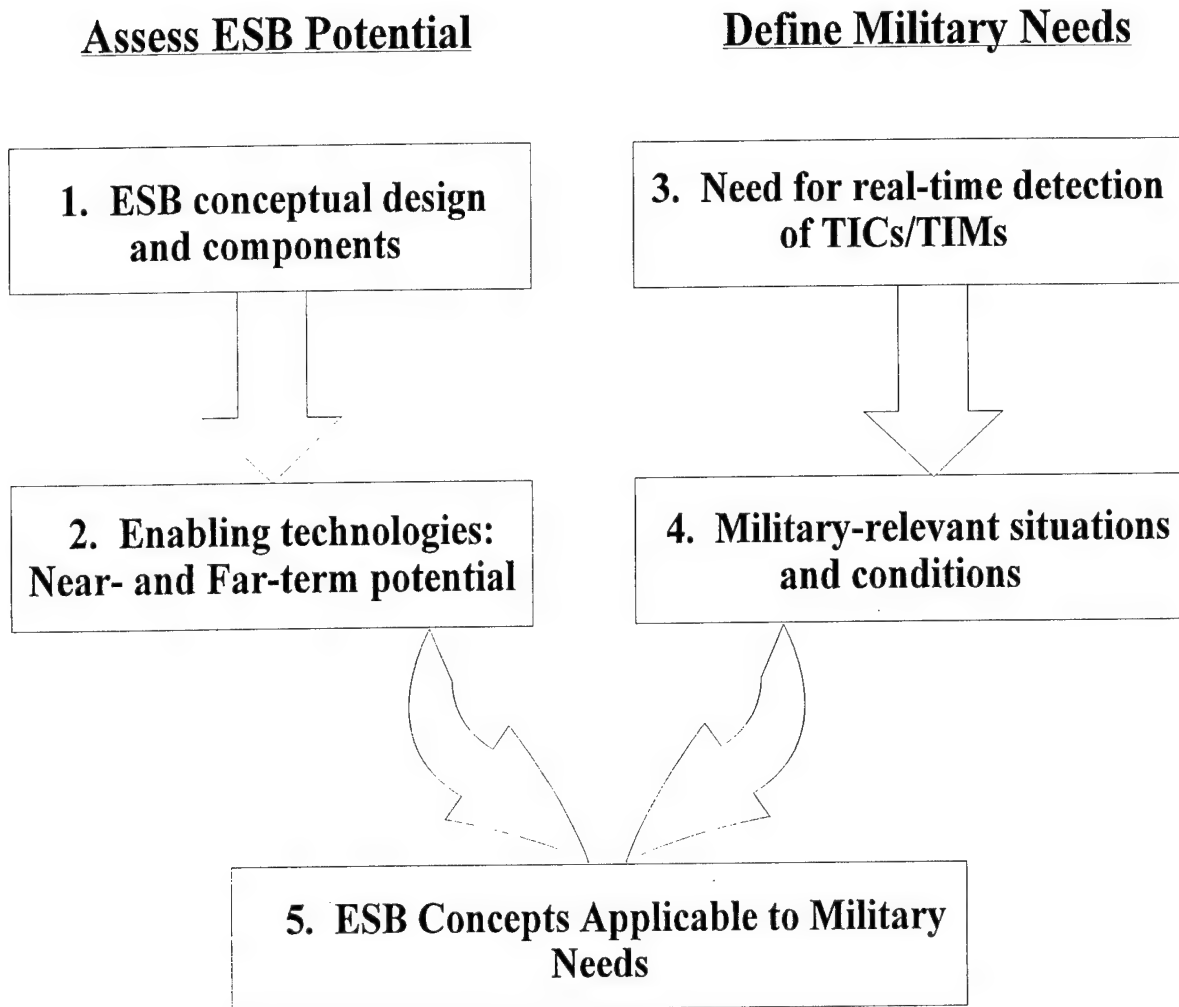


Figure 2. Overall Approach

- National Aeronautics and Space Administration
- National Institute for Standards and Technology (NIST)
- National Oceanic and Atmospheric Administration
- US Department of Agriculture
- US Department of Energy, National Laboratories
- US Environmental Protection Agency (USEPA)
- US Geological Survey

All comments and responses to the questions included in the Concept Paper are taken into consideration in this White Paper. A draft of this White Paper was peer reviewed by Mr. John Resta, USACHPPM, LCDR Ava Maria Conlin, NEPU No. 5, Dr. Joseph Pancrazio, US NRL and Dr. Robert Cartledge, Strategic Technology Decisions. The comments of the peer reviewers were taken into consideration in finalizing this document.

After independent peer review, this draft White Paper will be finalized and serve as the starting point for discussions at a 2-1/2 day Workshop scheduled for 9/26-28/01 in Frederick, Maryland. Plans are for this Workshop to bring together those who provided inputs to the White Paper, many of those who provided comments on and responses to the Concept Paper, the White Paper peer reviewers and other representatives of users' and developers' organizations identified throughout the project. Prior to the Workshop each participant will receive a copy of the final White Paper, the Workshop charge, and a set of questions. Responses to the questions will be solicited from Workshop participants prior to the meeting. The responses will be analyzed, presented, and discussed during the Workshop. The final product will be a Workshop Report based on written inputs from the Workshop chair, work group leads and selected Workshop participants. Since reaching consensus at the Workshop on the best ESB system is not an objective and is not expected, the Workshop Report will summarize all of the Workshop deliberations to assist the Workshop sponsor, USACEHR, and others on potential courses of action to pursue for Research and Development (R&D) of ESB systems for military field use.

ESB SYSTEMS CONCEPTS AND WORKSHOP FOCUS

- For this project, ESB systems are defined as:

Biologically-based systems (in vivo, in vitro, synthetic) and chemical sensors placed in the environment (in situ), either mobile or stationary, that can sense a biologically-significant event and provide relevant real-time data for use in risk assessment, mitigation, and/or management.

Real-time data are not limited to data available instantaneously to the user/decision maker. Data available within the time frame required by the military decision maker are considered real-time for this project. This is envisioned to typically be less than one hour.

The overriding questions the project is attempting to answer are:

1. Can ESB systems be useful to the Military's Force Health Protection (FFP) program?
2. If they can be useful, how can ESB systems contribute most to the military's FFP program? What types of data and information are needed? Under what military

conditions/scenarios should ESB systems be expected to operate?

3. Is research needed to better answer the two questions above? And if so, what scientific and technical challenges must be overcome and what data and information must be generated?

- The focus is on the scientific, not the engineering, aspects of integrated ESB systems, achievable within five years (referred to as near-term throughout this Paper) and those achievable within five to 10 years (referred to as far-term), with the potential to ultimately provide continuous, real-time monitoring of single known and unknown TICs/TIMs and their complex mixtures under anticipated military field conditions and operational scenarios integrating both biological effects and chemical measurements and possibly triggering supplemental efforts (e.g., sample collection and/or additional analyses).

Potential military field conditions span all future military missions and deployment types, include force structures ranging from small single units to large joint and multinational forces capable of fighting a major theater war and consider the wide variance in terrain and the weather extremes that may be encountered throughout the world.

For this project the following relative comparisons within performance parameters have been selected as a starting point for Workshop discussions and focus. The assigned relative importance decreases from left to right as indicated by the arrows:

- ☐ Remote operation → hands-on operation
- ☐ Adverse human health effects → exposure estimates
- ☐ Broad spectrum effectiveness → narrow spectrum effectiveness
- ☐ Real-time data → near real-time data → delayed data
- ☐ TICs/TIMs → CWAs → BWAs
- ☐ Chemical mixtures → single chemicals
- ☐ Integrated response over time → one-time response
- ☐ Severe adverse human health effects → moderate → mild → negligible effects
- ☐ Warning/alert to risk → documentation of risk without timely warning
- ☐ Cumulative, multi-pathway effects → instantaneous, single-pathway effects
- ☐ Field deployments → fixed military facilities
- ☐ Military unit/geographical location-relevant data → individual personnel data

Workshop deliberations may result in additional performance parameters and/or changes in relative importance.

- The emphasis is on endpoint selection and technologies for data acquisition, analysis and interpretation. Development of decision making algorithms is also considered critical to ESB systems usefulness. Enabling technologies for data dissemination, transmission, archiving, storage and retrieval while important to ESB systems success will not be addressed in detail in the White Paper or at the Workshop.
- Not included in the scope of this project are:

- ☐ ESB system applications limited to environmental quality protection: support for pollution prevention, hazardous site clean-up and remediation, environmental

- compliance, and conservation activities at military facilities.
- Laboratory-based screening applications that are not potentially adaptable to field monitoring by the military. Systems that are currently limited to laboratory use but appear to have the potential to be adapted to field use are to be considered and may represent some of the best near-term opportunities.
- ESB system applications specifically/exclusively for CWA and BWA detection, identification and quantification that are not capable of use in the monitoring of TICs/TIMs.

PROJECT OBJECTIVES

The objectives of this project include:

- Determining if ESB systems have the potential to satisfy certain materiel portions of existing and/or possible future Mission Need Statements (MNSs).
- Proposing critical system performance capabilities for ESB systems. These capabilities could ultimately be incorporated into a future Joint Operational Requirement Document (JORD).
- Evaluating potential ESB system alternatives/options/configurations conceptually taking into consideration:
 - The readiness level of the technologies and technology barriers
 - Potential performance parameters and capabilities for military field use
 - Applicability to military conditions envisioned by users during:
 - Major Theaters of War
 - Single small contingencies involving conflict
 - Sustainment and Support Operations (SASO)
 - Terrorism incidents (at home and abroad)
 - Accidental chemical spills/releases affecting military personnel's health at Continental US (CONUS) and Outside the Continental US (OCONUS) locations
- Assessing the potential of ESB systems to assist preventive medicine personnel at all Command levels assigned the task of providing health risk assessments to the Commander based on Occupational and Environmental Health (OEH) surveillance.

THE MANDATE FOR ENVIRONMENTAL MONITORING IN SUPPORT OF MILITARY DEPLOYMENTS

Presidential Review Directive 5 (PRD-5) requires that DoD identify and minimize or eliminate the short- and long-term health effects of military service, especially during deployments (including war) on the physical and mental health of veterans. The need to identify and consider risk from low-level

exposures to chemicals and/or radiation for military personnel is acknowledged in PRD-5 and in the National Research Council's "Strategies to Protect the Health of Deployed US Forces." There is emphasis in PRD-5 to measure, document, and archive individual exposures to occupational and environmental health agents.

DoD Directive 6490.2, "Joint Medical Surveillance," sets forth that it is DoD policy that medical surveillance shall encompass the periods before, during and after deployment and include monitoring environmental threats. DoD Instruction 6490.3, "Implementation and Application of Joint Medical Surveillance for Deployments," states the Commander in Chief and Joint Theater Force Surgeon shall deploy technically specialized units with capability and expertise in the conduct of surveillance for occupational and environmental illnesses, injuries, and diseases, health hazard assessments, and advanced diagnostic testing. These units shall conduct health assessments of potential exposure to biological, chemical, or physical agents that threaten the health and safety of the command.

An indication of the intent of Congress regarding protection of DoD personnel from toxic chemical exposures can be seen in the language of Public Law 105-261, Section 247. Paragraph (b) of Section 247 of this law requires DoD to review and modify policies and doctrines on chemical warfare defense in order to provide for the prevention of and protection against, and the detection of exposures to a CWA (whether intentional or inadvertent) at levels that, even if not sufficient to endanger health immediately, are greater than the level that is recognized under DoD policies as being the maximum safe level of exposure to that agent for the general population. Certainly the implication is to have protection equal to that estimated for the general population whenever and wherever possible for DoD personnel. DoD Instruction 6055.1, "DoD Safety and Occupational Health (SOH) Program," specifies that environmental monitoring and risk assessments for DoD personnel OCONUS be performed using the Military Operational Risk Management (ORM) process.

In the Army policy letter on FHP-OEH threats, commanders and other decision makers are required to:

- Strive to adhere to peacetime US or Host Nation environmental, safety and occupational health standards (whichever are more restrictive)
- Make risk decisions based on complete consideration of operational as well as OEH risks and available contingency guidance and criteria
- Document, archive and reevaluate OEH risks on a recurring basis
- Consider both short-term and long-term health risks to personnel from OEH exposures
- Use the ORM process to minimize the total risk to personnel

Existing service policy and doctrine already establish a framework for military commanders to use in making ORM risk decisions.^(a) The Services' ORM guidance documents have similar content. They state the principles of risk management, use a stepwise process, and include a risk assessment matrix. In the risk assessment matrix, the risk level element (either extremely high, high, moderate or low) is determined at the intersect of the hazard severity row (either catastrophic, critical, marginal or negligible) and hazard probability column (either frequent, likely, occasional, seldom or unlikely). This is depicted in Table 1.

(a) Key documents that provide ORM guidance include US Army Field Manual 100-14, *Risk Management*, Air Force Instruction 91-213, *Operational Risk Management (ORM) Program*, Air Force Pamphlet (AFPAM) 91-215, *Operational Risk Management (ORM) Guidelines and Tools*, and OPNAV Instruction 3500, *Operational Risk Management*.

Table 1. Risk Assessment Matrix

Hazard Severity	Hazard Probability				
	Frequent	Likely	Occasional	Seldom	Unlikely
Catastrophic	E	E	H	H	M
Critical	E	H	H	M	L
Marginal	H	M	M	L	L
Negligible	M	L	L	L	L

E = Extremely High Risk
H = High Risk

M = Moderate Risk
L = Low Risk

Technical Guide (TG) 248, "Guide for Deployed Military Personnel on Health Risk Management," which is in final draft form, provides a general understanding of the process to be used for OEH hazard assessment in the Army's ORM decision-making. Its primary objective is to enable commanders to make decisions on OEH risks and to put them in the same context as other operational risks.

In the draft of TG 248 both medical and health threats are considered. Medical threat is defined in service publications as a composite of all ongoing potential enemy actions and environmental conditions and disease and non-battle injuries that may degrade a unit's effectiveness. Health threat refers to an individual's health. The term can include hereditary conditions, individual exposure to TICs/TIMs where others in the unit are not exposed, or conditions that can result in other injuries and traumas that affect an individual's health but may not affect the health of the military unit. Units that experience 40 to 50 percent of their personnel exhibiting a debilitating condition cannot complete their missions. Health threats of greatest concern to the Command have been those health threats that are of immediate importance to the Commander based on the nature of the operations and/or related considerations.

The following definitions are included in TG 248:

- OEH Hazard Probability - The magnitude, frequency and duration of exposure of unit personnel to health threats integrated with the expected incidence of exposure within the unit relative to guideline levels.
- OEH Hazard Severity - The potency of the hazard to cause injury, illness, disease, adverse health conditions, or death integrated with the significance of the health consequences for personnel relative to the ability of the field unit to complete the mission or maintain readiness.

The emphasis on environmental surveillance has increased greatly since and probably because of Gulf War Illnesses coupled with the great increase in SASOs involving large numbers of personnel (military and essential DoD personnel and DoD contractor personnel) for assignment durations up to one year. Deployed military frequently now include Reserve and National Guard personnel.

MISSION AND THREAT ANALYSIS

There is a need for the joint forces to effectively operate across the continuum of global, contingency operations including:

- special operations/low intensity conflict
- smaller-scale combat operations
- counter proliferation
- multilateral peace operations
- counterdrug
- counterterrorism
- sanctions enforcement
- noncombatant evacuations
- humanitarian and disaster relief operations
- regional conflict
- major theaters of war

There has been and continues to be a significant military effort to enable forces to operate safely, survive and sustain operations in Nuclear, Biological and Chemical (NBC) agent environments. Until recently there has not been a concomitant effort by the military associated with potential environmental exposures during deployments to TICs/TIMs and other stressors not identified as causing adverse health effects or operationally relevant performance decrements to DoD personnel during the deployment. While solutions for NBC defense offer potential spin-off to certain TIC/TIM situations, there are significant differences.

A number of factors have raised concern about TIC/TIM environmental and occupational exposures for deployed personnel. Included are:

Leading Factors

- The uncertainties relative to Gulf War Illnesses
- The mandate to identify and consider risks to deployed DoD personnel from low-level exposures that may produce delayed and/or chronic health effects to male and female DoD personnel including regular, reserve and National Guard personnel, DoD civilians and deployed DoD contractor personnel.

Secondary Factors

- Worldwide increase in production, transport, distribution, storage, use and disposal of TICs/TIMs
- Continued introduction of new TICs/TIMs into commerce
- US deployments to urbanized and industrialized areas
- Lax security at industrial complexes in theaters of operation
- Environmental quality and public health protection and practices in certain deployment areas significantly below US standards and practices
- Degraded infrastructure (facilities, equipment and operation and maintenance) in certain deployed areas
- Limited data on the long-term adverse health effects of low-level chemical exposures to many TICs/TIMs, including reproductive effects.
- Recognition that typically TIC/TIM exposures are not to a single chemical and by a single exposure pathway for a well-defined, finite duration, pattern and frequency but to mixtures of chemicals with many and varied exposure durations, patterns and frequencies and multiple exposure pathways

- Recognition that other non-TIC/TIM stressors in combination with TIC/TIM exposure can confound prediction of the adverse health effects and need to be taken into account
- Continuing use of certain chemicals in some foreign countries that are no longer produced in the US and used in the US, such as dichlorodiphenyltrichlorethane (DDT).

Operational environments for joint forces are becoming potentially more dangerous due to the increased number, use, and misuse of toxic and hazardous chemicals across the entire range of military missions. Defense personnel may be exposed to harmful chemicals as a result of industrial accidents or intentional or unintentional action of enemy, friendly forces, or indigenous populations. Table 2 categorizes these potential chemical exposures of deployed personnel. Table 3 is a comparison of a number of factors that differ for CWAs and TICs/TIMs and impact the military's needs. The differences between CWA and TIC/TIM appears to make it unlikely that a system designed to satisfy the military's needs for either CWAs or TICs/TIMs will fully satisfy the other; however, some of the same technology may have application for both and combined platforms for TICs and CWAs would be desirable.

Table 2. Potential Chemical Exposures of Deployed Personnel^(a)

Threat Category	Scenario	Plausible and Past Examples
Intentional	Chemical warfare agents against US forces: known agents and unknown agents synthesized	Prevalence of chemicals and ease of synthesis: Iran-Iraq War, Gulf War threat
	Direct "poisoning" of resources by enemy forces or terrorists (air, water, soil, or food)	Persian Gulf oil fires, dumping pesticides in water supplies, ignition or pressurized release of fuels and industrial chemicals and munitions
	Collateral, intentional friendly forces emissions, discharges, etc., into the environment	All intentional, unavoidable releases from all military operations during deployment
Unintentional	Accidents and mishaps that release quantities of toxic substances, by-products or decomposition products into the environment	Bhopal-type disasters, transportation accidents, spills and leaks from equipment or weapon systems (Pyrotechnics, explosives, hydraulic fluids, fuels, refrigerants, fire suppressants, etc.), firefighting during damage control at industrial facilities
	Mission/job related exposures during combat operations, training, deployments and maintenance support activities by troops	Hand-held or mobile weapon systems releasing chemical contaminants and by-products [Agent Orange exposures, confined space exposures (ship, sub, tank, aircraft)]
	Environmental exposures from non-military activities causing pollution in area	Air and water pollution, hazardous waste sites, contaminated soils and foods, black market dumping of hazardous wastes
Intentional and/or Unintentional	Combinations of several of above	All of the unintentional threat categories are likely to be experienced during deployment to some extent

(a) From *Deployment Toxicology Research & Development Master Plan*, September 1997.

Table 3. Comparison Between CWAs and TICS/TIMs

Factor	CWAs	TICs/TIMs
Number of Potential Agents	Relatively short list of weaponized agents.	Approximately 70,000 industrial chemicals with about 1,000 new chemicals introduced each year.
Likelihood of Being Encountered Individually	Use of a single type more likely than simultaneous use of multiple CWAs.	Greater potential for exposures to mixtures.
Exposure Duration	Temporary or short unprotected exposures are considered more likely than long-term exposures.	Exposures of all durations (temporary, short and long term) can be encountered.
Extensiveness of Threat	Small but growing number of countries and non-nation groups have capability to either produce or deliver CWAs and use them. Long recognized as an acute health threat to military personnel.	Production, transport, use and disposal of TICs/TIMs in increasing number and quantities generally associated with expansion of industrialization and agricultural practices using pesticides and chemical fertilizers. More recent concern of TIC/TIMs as short- and long-term threat for military personnel, particularly during deployment.
Exposure Frequency	One-time unprotected exposure more likely than multiple exposures to same CWA by deployed personnel.	Repeated exposures during a deployment somewhat more likely, particularly if both source and exposed population are stationary over extended length of time.
Military R&D Investment in Sensors, Detectors, Alarms and Monitors	Long standing military R&D program and substantial amount of fielded equipment.	Limited, more recent military R&D program and fielded equipment specifically for TIC/TIMs limited to a few kits.

Permanent US military installations, OCONUS and in the CONUS, also are potential targets for terrorist acts employing TICs/TIMs and could experience an on-base or off-base accidental TIC/TIM spill/release.

Since the Gulf War ended, nonconflict deployments involving more than 1,000 US troops/deployment total more than 50. Currently more than 250,000 US troops are serving overseas, either aboard ships or in 145 countries and US territories. Major OCONUS troop locations and approximate numbers of deployed personnel are provided in Table 4. Reserve, National Guard and active-duty personnel are included in these totals. The reserve military now spends an average cumulative total of 13 million days on active duty per year. This is an increase of about 13 times the rate prior to the Gulf War.

Table 4. Deployed Locations and Strength

<u>Country</u>	<u>Approximate No. of Deployed Troops</u>
Belgium	1,500
Bosnia	5,700
Germany	69,000
Iceland	1,600
Japan	40,000
Portugal	1,100
Puerto Rico	2,900
Saudi Arabia	7,000
Serbia	5,200
South Korea	36,500
Spain	2,000

The TIC/TIM hazards are addressed in several recent military documents:

- Defense Intelligence Assessment DI-1651-1-00, Military and Terrorist Use of Toxic Industrial Chemicals and Materials (U), Sept 99 (Classified);
- Defense Intelligence Report DI-1816-8-99, Medical Intelligence Assessment of Deployment Environmental Health Risks, Jan 99, (Unclassified);
- International Task Force (ITF)-25: Hazards from Industrial Chemicals Final Report, Mar 96, (Unclassified); and
- USACHPPM TG 230 on chemical exposure guidelines for deployed military personnel.

When viewed in total, these documents provide some insight to the diversity and enormity of the potential TIC/TIM hazards military personnel may encounter while deployed or in garrison. The exposures experienced by the military during past deployments may not be good predictors of future exposures because each deployment is likely to bring with it some new environmental TIC/TIM-related threats. The ITF-25 report ranks 98 toxic chemicals using a hazard index approach into high, medium and low categories. All of these chemicals are produced in quantities exceeding 30 tons/yr at at least one plant, have a LC₅₀ value by inhalation in mammalian species of less than 100,000 mg min/m³, and an appreciable vapor pressure at 20 C and/or are listed in the US Department of Transportation Emergency Response Guidebook.^(a) The ITF-25 hazard rankings are applicable only to airborne exposures. An ongoing effort, ITF-40 will expand on ITF-25 by considering all routes of exposure and additional hazards, such as flammability and corrosivity. This task force has not yet published its hazard rankings.

Although not specifically targeted at TICs/TIMs, there is a NBC Joint Future Operational Capabilities (JFOC) for contamination avoidance that requires a significant improvement in tactical, operational and strategic NBC situational awareness by rapidly detecting, locating, identifying, quantifying, confirming and disseminating TIC/TIM detection and identification to the Joint Force. The chemicals must be

(a) US Department of Transportation, 1993 Emergency Response Guidebook, ISBN-0-16-042938-2.

detected and identified in the vapor, solid or liquid form, including diluted in water and aerosols. Sampling must be of adequate quantity, quality, and stability to provide evidence to the national command authority. Confirmation of enemy use of NBC weapons against US forces is vital information for use in determining US military retaliation as well as defense against NBC.

There are draft MNSs for hazards from industrial chemicals and for FHP against NBC and TIMs. Contamination avoidance shortfalls cited in the draft MNS for hazards from industrial chemicals are:

- The absence of reliable, effective, real-time TIC/TIM detection capabilities against the full range of TIC/TIM threats at sensitivity levels associated with immediate and long-term physiological effects. There is no technology to provide for automated point detection of TIC/TIM agents in water, human body fluids, or on surfaces.
- This MNS also ranks the high priority chemicals identified in ITF-25 in order of their importance as threats to deployed personnel. The ranking is included in Appendix 1. Since only the chemicals in the ITF-25 report were considered in this MNS, the ranking only applies to inhalation exposures. The following six factors are used:
 - Production, Storage and/or Transportation Quantity
 - Toxicity
 - Vapor Pressure
 - Effectiveness of Current Military Protective Equipment
 - Detection by Smell and Sight
 - Special Information, such as past use as a weapon, the chemical's reputation and unexpected chemical behavior

The JFOC for Medical NBC Defense includes the ability to rapidly identify, report, and document NBC-Environment/TIC/TIM threats through laboratory analysis in theater to provide medical situational awareness and decision support to the commander. Included in this capability is rapid and automated disseminations, recording and archiving of medical surveillance reports and analyses.

There is greater attention in these military documents to airborne TICs/TIMs than other exposure pathways. However, USACHPPM's technical guidelines, TG 230 includes a large number of chemicals in water as well as air. TG 230 also includes guidelines for chemicals in soil. There are no short-term exposure guidelines in TG 230 for soil because acute health hazards are considered a negligible concern for toxic chemicals in soil. In TG 230, the chemicals are grouped into high, medium, low and unknown priority groupings based on their toxicity and their level of use/production/environmental contamination.

Inhalation exposures are considered the route most likely to result in immediate, severe health effects to significant numbers of deployed military personnel. The drinking water troops receive in the field usually will have undergone treatment by the military's tactical water purification equipment, be obtained from an approved municipal source or it may even be bottled water. In emergency situations, individuals and small units may have to rely on iodine tablets, boiling, Chlor-Floc®, or chlorine ampules to disinfect water. Disinfection may be preceded by use of individual, hand-held treatment devices.

The current draft version of Technical Bulletin, Medical (TB Med) 577, Sanitary Control and Surveillance of Field Water Supplies, includes the US Tri-Service, Quadripartite Armies

Standardization Agreement (QSTAG) 245 and Standard North Atlantic Treaty Organization Agreement (STANAG) 2136 short-term (less than 7 days) and long-term (less than 1 yr) field water quality standards. These standards were developed to protect against performance-degrading effects resulting from the ingestion of field water.

The Tri-Service standards include the following six chemical entities:

- arsenic
- cyanide
- chloride
- lindane
- magnesium
- sulfate

There is no QSTAG or STANAG standard for lindane. There are QSTAG and STANAG standards for the other five chemical entities.

The three standards also include physical properties (color, odor, pH, temperature, Total Dissolved Solids (TDS) and turbidity) and include short-term but not long-term standards for some CWAs.

TB Med 577 also lists the USEPA's national primary drinking water regulations and states they should be adopted as a goal to provide the highest quality field drinking water possible.

Although the military's tactical water purification equipment is very effective in producing potable water when maintained and operated properly, some TICs not included in the US Tri-Service standards could be present in product water in concentrations representing both short- and long-term health risks.

To assess prospective field water sources, TB Med 577 requires that tests for the US Tri-Service water quality standards be conducted to determine if the source can be made potable by water purification. The TIC/TIM waterborne threat must also take into account the potential for post-treatment contamination during storage or transport. Deliberate contamination of water after treatment is likely to be more effective as an act of terrorism attempting to produce casualties than contamination of surface or ground water supply sources that undergo purification. Contamination of a field water supply source with TICs, however, can affect production quantities, damage water purification equipment or force relocation of the water point.

POTENTIAL ESB SYSTEM PERFORMANCE PARAMETERS AND KEY OPERATIONAL CONDITION DESCRIPTORS

The JORD for the Joint Service Agent Water Monitor (JSAWM) has as an objective detection, identification, and quantification of all TICs, agricultural chemicals, and biological pathogens harmful to personnel in addition to CWAs and BWAs. System performance parameters for the JSAWM provide some indication of threshold and objective performance parameters for ESB systems. The draft MNS for hazards from industrial chemicals does not list detailed performance parameters but cites some constraints for TIC/TIM agent defense capabilities.

Listed below are system performance parameters and key operational condition descriptors developed from review of the JSAWM JORD and the MNS for hazards from industrial chemicals. Only parameter conditions that appear appropriate to consider at the "proof-of-concept" stage are included.

System Performance Parameters:

- Physical/Environmental Requirements
 - ☐ Rugged, small and lightweight
 - ☐ Modular components for different scenarios
 - ☐ Man portable system preferred to transportable system, particularly when used in support of smaller size force
- Logistic Requirements
 - ☐ Rapid, efficient, simple, operator friendly start-up/shut-down, operation and maintenance
 - ☐ Few supplies
 - ☐ Long operational life
 - ☐ Safe to operate
 - ☐ Easily transported by air, land and sea and easily stored
 - ☐ Long shelf life
 - ☐ Small logistical footprint
- Detection Requirements/Features
 - ☐ Provides a significant new capability to the warfighter not just a replacement or marginal improvement for an existing capability
 - ☐ Able to identify, detect and quantify or be used effectively for a large number of TICs/TIMs and TIC/TIM classes of compounds
 - ☐ Few interferences
 - ☐ Capable of being upgraded over time
 - ☐ Have a high probability of detection (at least 0.95) at desired levels of sensitivity
 - ☐ Few false positives and negatives, high reliability
 - ☐ Simple calibration or self-calibration
 - ☐ Self-diagnostic troubleshooting feature or self-test feature
 - ☐ Quantify and record environmental conditions, such as temperature and humidity
 - ☐ Discriminability in presence of contaminants
 - ☐ Ease of operation
- Data Output Requirements
 - ☐ Provide a digital or text readout
 - ☐ Provide a recoverable data record
 - ☐ Link and be interoperable with military systems for CWA/BWA warning and the Joint Technical Architecture

Operational Condition Descriptors:

- Compatible with existing and planned command, control, communications, computer and intelligence interfaces
- Capable of operations in all climate conditions and terrain
- Capable of operation in support of all types of military missions
- Have NBC survivability

The relative priority for monitoring TIC/TIM exposures and/or their effects on DoD personnel during a specific deployment and allocating resources for such activities generally increases:

- As conflict intensity and/or threat of conflict decreases
- As duration of a deployment lengthens
- As number of personnel deployed increases
- As threat of terrorist or enemy acts involving TICs/TIMs increases
- When level of industrialization in deployed/theater of operations increases
- As infrastructure degradation increases
- When base camps are established and maintained for deployed forces
- When environmental standards of area are low or non-existent and environmental controls, treatment and disposal practices are below US standards

CURRENT FIELD MILITARY CAPABILITY FOR TIC/TIM DETECTION, IDENTIFICATION AND QUANTIFICATION DURING DEPLOYMENTS

The US military does not have a deployable ESB system for TICs/TIMs. The services rely heavily on commercial analytical chemistry equipment for TIC/TIM detection, identification and quantification. Most of the detailed chemical analyses are conducted on discrete environmental samples that must be performed in a field laboratory setting by a trained technician. In some situations, samples must be shipped to permanent laboratories out of the theater of operations for testing. Typically there is a considerable lapse in providing results to decision makers when this type laboratory testing is required.

Water purification teams are responsible for operational monitoring of the production of field water to ensure the efficacy of water purification equipment and the treatment process. These units utilize the Water Quality Analysis Set - Purification (WQAS-P) to measure pH, temperature, turbidity and TDS. The WQAS-P uses electrochemical detection analysis and has a battery of probes that provide data to water purification specialists to evaluate operation of purification equipment, ascertain the performance of water purification equipment and safeguard the equipment from potential damage because of influent water quality. The WQAS-P is the replacement for the Water Quality Analysis Set - Engineer (WQAS-ENG). Although the WQAS-ENG is no longer issued, some of the sets are still in use. Additionally, chlorine residual is to be monitored by the waterpoint operator at the point of production 30 minutes after contact.

Monitoring of field drinking water supplies to approve water as potable is the responsibility of a variety of military medical organizations, including Army Preventive Medicine (PM) Detachments, Navy Environmental and PM Units, Air Force Bio-Environmental personnel, veterinary units and medical laboratories, such as the TAML. In addition to assisting in the inspection and selection of raw water sources, PM personnel certify the potability of product water, ensure maintenance of the required

chlorine residual to the point of consumption, and perform periodic sanitary inspections of water system(s) equipment. Samples are also collected for more thorough water analysis performed at fixed laboratories or other approved in-country or overseas facilities. Field medical units use the Water Quality Analysis Set - Preventive Medicine (WQAS-PM) and the Water Quality Test Kit, Chemical Agent (M272 Kit) and supplemental equipment to perform the health monitoring mission. Veterinary units inspect and approve Host Nation bottled water production facilities. Generally, these inspections will examine the sanitary conditions at a facility with samples collected for laboratory analysis.

The WQAS-PM has simple and rapid colorimetric (spectrophotometric, titration and color matching) tests to measure water quality. Different reagents and tests are used for the water constituents that the kit measures. In addition to containing tests that have no US Tri-Service field water standard, some tests cannot detect to current standards.

Commercially procured test strips supplement the WQAS-PM to enable personnel to test for arsenic, chloride, cyanide, magnesium and sulfate. The test strips are color matching tests and enable PM personnel to rapidly test (from one minute for chloride to 30 minutes for arsenic) for these five constituents.

A commercial bacteriological membrane filter test kit, either a Millipore or Hach product and the defined substrate test, Colilert or Colisure, are used in conjunction with the WQAS-PM to test for the presence of colony-forming indicators of fecal contamination in drinking water.

The M272 water quality test kit, chemical agent, fielded in the mid 1980's, is used to determine chemical agent concentrations in water through colorimetric, antibody-regulated changes in under 10 min. The M272 provides field water characterization for chemical agents at select concentrations only, and the kit has no capability to characterize incapacitants or T-2 toxins. Additionally, the kit is difficult to operate while in Mission-Oriented Protective Posture Level 4. A R&D effort is underway to replace the M272 with the JSAWM.

The AN/PDR 27 radiac set is an old item of equipment which is undergoing replacement. Although not specified as a water quality test kit, it can be used to characterize the radiological quality of water. The AN/PDR 27 provides a capability to measure beta and gamma radioactivity in water. The AN/PDR 27 replacement is the AN/VDR-2 which provides both beta and gamma detection. The AN/PDR-77 is also available. At this time it is fielded to units supporting storage and movement of nuclear weapons, facilities and materiel. The AN/PDR-77 is a modified version of the AN/VDR-2 with newer measurement electronics, and alpha and X-ray probes in addition to beta and gamma probes.

The chlorine comparator is a rapid (one min), simple colorimetric test that measures free available chlorine in field water. It is used in conjunction with both the WQAS-PM and WQAS-P. In the test, a water sample reacts with reagent, providing a sample that is matched and compared to known color/concentration measurements. There are comparator kits that include a pH capability because the effectiveness of chlorination is pH dependent. The N,N-diethyl-p-phenylenediamine method is preferred for measuring chlorine residual in field water supplies. Kits manufactured by Wallace & Tiernan Company and LaMotte Chemical Products Company have military stock numbers.

Although equipment sets and capabilities vary by service, the best capability available to the service's forward medical laboratories for chemical analysis of water is portable Gas Chromatography (GC). The GCs can conduct a range of analyses for organic chemical contaminants. Additionally, forward

laboratories also have test capabilities similar to those in the WQAS-PM kit (simple colorimetric test for specific analytes) as well as the Colilert test kit for microbiological testing. In addition, the Army medical laboratory has commercially produced Polymerase Chain Reaction (PCR), enzyme-linked immunosorbent assay and fluorescence microscopy test capabilities.

The focus of DoD's past R&D on CWA and BWA detection and monitoring equipment and systems has been on protecting personnel from short-term exposures to levels capable of causing adverse health effects to personnel during the mission. The military's field equipment for CWA detection and monitoring ranges from simple systems, such as detection paper, to vehicle-mounted detection systems. There are CWA detection kits, such as the M256A1 and M256A2 which contain detector "tickets" and/or disposable sample detection tubes. There is the M8A1 automatic chemical agent alarm for point detection of nerve agent vapors or inhalable aerosols. It employs ionization methods in a baffled flow electrode configuration. There is the Individual Chemical Agent Detector (ICAD) based on electrochemical techniques. There are stand-off chemical alarm systems that detect chemical agent vapor clouds from a distance; and there are point monitoring devices, such as the Improved Chemical Agent Monitor (ICAM) and the ICAM - Advanced Point Detector (APD). Both the ICAM and ICAM-APD simultaneously detect nerve and blister agents in vapor and aerosol forms.

The military's investment in R&D for field BWA detection systems has been considerably smaller than R&D for CWA detection and the available systems are less mature. The military has the Biological Integrated Detection System. It is a collection of components that provides mobile detection capability that includes an air sampler and staged compactor to concentrate aerosol particles. The Navy has the Interim Biological Agent Detector (IBAD) for shipboard use. The IBAD has a particle size sorter/counter, a wet cyclone sampler, a manual identifier and a membrane colorimetric ticket. The long-range standoff detection system, the XM94, detects aerosol clouds using Light Detection and Ranging (LIDAR) but does not explicitly detect BWAs.

Integrating chemical and biological sensors on an ESB platform should provide detection capabilities that chemical sensors alone do not have, such as:

- Identifying potential toxicity from unsuspected chemicals
- Integrating the toxic effects of multiple chemicals and multiple pathways
- Identifying need for further chemical analysis based on biological responses
- Confirming toxicity suspected based on chemical analyses

ESB CONFIGURATIONS AND APPLICATIONS AND ENABLING TECHNOLOGIES

However one conceptualizes ESB systems, they all undoubtedly will involve a battery of different type biosensors and most likely involve some online analytical chemistry components. Figure 3 (adapted from *Deployment Toxicology Research and Development Master Plan*, September 1997) provides a depiction of ESB systems' potential role in FHP.

Configuration and application for the near- and far-term need to take into account component integration, possible applications, sampling strategies and system placement. Each is elaborated on below.

- **Component Integration:** Use a battery of different type biosensors in combination with some online analytical chemistry components.
 - Systems that use multiple components to provide confirmatory results are preferred to single sensor systems in order to reduce false positives/alerts
 - Use whole organisms to detect developing toxicity based on physiologic responses, in combination with cell, tissue, and/or chemical-based biosensors and/or gene microarrays targeted to analytes or groups of analytes can produce real-time data that, with proper analysis, is useful to operational decision-making
 - It may be possible to use biological/physiological information to help determine causality (e.g., toxicant mode of action)

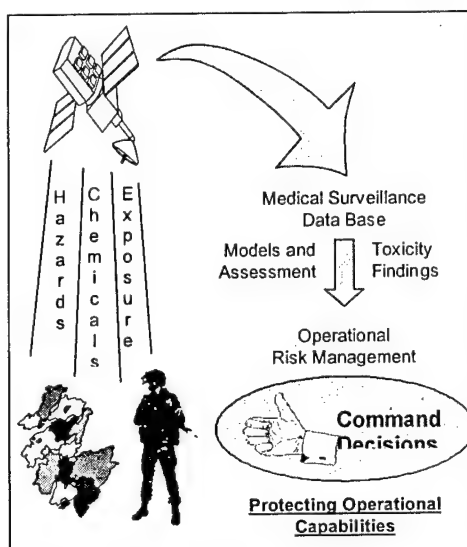


Figure 3. ESB Systems' Potential Role in FHP

- System response. Response to acute versus chronic effects - may detect possible long-term health effects associated with exposures that do not produce clinically/immediately observable adverse health effects in deployed DoD personnel. There may be a trade-off between increased sensitivity and decreased reliability (e.g., false alarms).
- **Possible Applications**
 - For each deployed individual or for specific deployed individuals within a unit considered to be representative of the unit
 - Near-term: Appears infeasible
 - Far-term: A small device for each deployed individual to wear or for selected individuals of a unit to wear. Dosimeters, such as those used for radiological monitoring, present an attractive concept. May provide both a warning of approaching

conditions requiring action (e.g., avoidance, protection) and a record of exposure. Another concept would be impregnated uniforms with different reactive chemicals which would change color when medical work-up is required.

- ☐ For mobile military unit or equipment activities
 - Near-term: ESBs mounted in vehicles, such as the "Fox", or in trailers
 - Far-term: ESB mounted Unmanned Vehicles
- ☐ For geographical location or area or water body
 - Near-term: Fixed location placed by ground personnel or air dropped, perimeter posturing or self-propelled biosensors, used with global positioning capabilities. Transmit signals to either a ground, air, or space-based receiver/processor.
 - Far-term: Network multimedia sensors combining data from all three types of applications
- Sampling Strategy and ESB Placement
 - ☐ Point source representation of larger areas.
 - ☐ Tailor ESB systems in the field to the military situational analysis. Use available intelligence information to determine potential environmental threats and select from an inventory of biosensor capabilities the sensors and their configuration for the mission. This could be a baseline capability and "plug-in" supplemental sensors that augment to baseline capability.
 - ☐ Biologically-directed sampling. Have biosensor responses activate additional biosensors and/or online analytical chemistry equipment either for validation or for specificity or refinement.
 - ☐ ESB placement must also take into consideration the physical security needs of the ESB system and the security of any data that is generated by ESB systems.
- Platform Technology
 - ☐ fixed platforms
 - ☐ mobile platforms (trailers, manned and unmanned vehicles, etc.)
- Data Integration and Analysis
 - ☐ chemical mixture issues
 - ☐ use of effects components to trigger sample collections, sample preparations and chemical/biological sampling analysis
 - ☐ use of effects and exposure information to assess health risks
 - ☐ interpretation of effects component responses

■ Major Challenges

- Validation and interpretation of effects and chemical/biological analysis components relative to adverse human health effects and functional performance decrements
- Effects component responses to non-toxic variations in environmental conditions
- Sensor stability in long-term operation
- Potential military allocation of resources to ESBs (logistics load, communication and computer capacity, personnel)
- Methods to handle uncertainty

An issue any new method faces is validation in comparison to accepted methods. For exposure measurements the comparison can be straightforward, but for adverse human health effects it is more complex, costly, and time consuming. Advances in environmental toxicology testing, modeling, simulations and medical diagnostics and treatment impact data needs and use.

Another across-the-board issue is the effect of interferences in the air, water, or soil that bias results and contribute to substantial error. The issue of liability cannot be ignored. What is the military obligated to report, to whom and when? This includes situations involving US forces, multi-national forces and non-combatants in the TO and TIC/TIM sources that could be generated by US military activities or the current or past activities of others.

This section contains the submissions of five subject-matter experts on enabling technologies. Each provided a write-up on a different enabling technology for field military ESB systems. Dr. Melvin Andersen on cell/tissue-based biosensors, Dr. William Waller on whole organism biosensors, Dr. Wayne Bryden wrote on online analytical chemistry, Dr. Amanda Jenkins on chemical-based biosensors, and Dr. Joanne Andreadis on Deoxyribonucleic Acid (DNA)-based microarray detection. The technical content of their inputs is unchanged from what they submitted.

Cell/Tissue-Based Biosensors by Dr. Melvin E. Andersen, Colorado State University

Abstract

The military services require a group of environmental sentinel biomonitors (ESBs) to assist in evaluating the healthfulness of various deployed environments. These ESBs may cover a wide range of systems from molecules to intact organisms. This short overview discusses the possibilities for development of cell and tissue ESBs for these applications. ESBs may be used in several contexts. They may be employed to evaluate the presence of known chemical risk factors in the environment. Here they might serve as quantitative tools to assess the concentrations of these compounds in the environment. Coupled with knowledge of the biological activity of specific compounds, data derived from these ESBs permit prediction of the short-term and longer-term health risks from exposures to measured levels of the specific chemicals. More importantly, ESBs, used in combinations covering multiple levels of biological organization, may be applied to evaluate the "healthfulness" of the environment, assessing possible adverse responses to real world mixtures containing some number of unknown compounds. This latter application, identification of possible adverse consequences without clear knowledge of the toxic compounds, needs to be embedded in a larger strategy to assess the

consequences of environmental exposures. This strategy would include continuing studies to identify constituents and toxicity studies in the integrative test systems and intact animals. The identification of exposure conditions that cause adverse responses in cell and tissue ESBs where the relationship of toxicity with the agents in the environment is unclear may also signal a need for more extensive epidemiological follow-up of exposed military populations. This paper outlines the various uses of cell and tissue based ESBs for exposure and bioeffects monitoring and notes how these ESBs fit into this larger strategy for both short-term and longer-term risk assessments for personnel in deployed environments.

Definition of Task

This paper discusses application of ESBs based on cells and tissues and the way they could be implemented within a strategy to assess both short-term and longer-term health risks associated with deployed environments. Cell based ESBs could be based on "natural" cells derived from specific biological organisms or represent "engineered systems" designed by adding or deleting specific genes or suites of genes to derive cells with specific biological functions as ESBs. Tissues, in this paper, refer to specific aggregates of cells with functions derived from the aggregation of multiple cell types. The revolution in molecular biology (actually in biology itself) over the past twenty years permits creation of a wide variety of engineered cells with genetic cassettes that allow response to various external stimuli. These responses might be related to exposure to a particular compound or a structurally related set of compounds or they might be designed to assess a cellular response to an integrated characteristic of the compounds in the environment, such as induction of markers of oxidative stress, DNA repair, etc. Both types of cellular ESBs, specific to a chemical compound or generic to properties of the environmental milieu, have potential value in deployed environments.

Tissue ESBs could also assess integrated functions disrupted by exposures to TICs. An example of a tissue system might be a culture of heart cells with rhythmic beating characteristics that could be disrupted by TIC exposures. Cell and tissue ESBs have a great potential for monitoring in deployed environments, although challenges remain in defining the cell systems to be developed for use, in automating their field use, and in interpretation of acquired data in relation to risk assessment for short and long-term responses of military personnel. The task in this paper was to outline how cell and tissue ESBs might be used in assessing the short and longer-term risks of deployed environments. To achieve this goal a portion of the paper serves to discuss the overall goals of biomonitoring programs and how cell and tissue based ESBs may serve to achieve these goals.

Overview of Paper

While this paper specifically focuses on the application and potential development of cellular and tissue ESBs, it is important to appreciate the context in which any of these ESBs might be used and the goals of their use. In this regard, the paper begins with a discussion of the expected application of the data derived from ESBs in general and from cellular and tissue ESBs in particular. These design goals include identification of the level of specific chemicals or groups of chemicals in the environment and/or measurement of the overall "healthfulness" of the environment. In the latter case the ESBs serve to integrate possible biological responses in the absence of knowledge about causation by specific chemicals in the environment. The short term risk assessment needs are more for rapid identification of known compounds that pose a risk or for evaluation of toxic responses in specially designed biomonitoring systems to determine if the exposures carry any "unusually high" order of risk. For risk assessments related to more chronic effects, the same issues arise in a slightly different context.

In devising strategies for cell based ESBs, there should also be concern for exposure to known compounds where long-term effects might arise in pulmonary or nervous system function or in relation to neoplasia and cancer. There are also health concerns about what might occur in human populations after exposures to environments where the integrated monitors show clear adverse responses, but analytical and other tools fail to discern the specific causally related exposures. To fit the variety of ESBs into this contextual structure, this paper uses a strategy, with associated flow chart for data collection and interpretation of results from analytical studies and ESB responses that lead to specific roles for ESBs in this strategy. Following this organizational discussion, the state-of-the-art in cell and tissue ESBs are discussed in relation to their potential contributions to short and long term health risk assessment/surveillance and in relation to the nature of cell and tissue ESBs that are available today and might be available in the future.

Biomonitoring in Deployed Environments

Health monitoring for any occupational environment includes assessments of contaminants in the atmosphere, evaluation of tissues of exposed populations for biomarkers of exposure or of response, and possible surveillance/epidemiology on the workers. Similar activities are required in assessing the risks to military personnel in deployed environments. The military is considering use of ESBs representing different levels of biological organization – molecules, cells, tissues, organisms, etc. These ESBs are intended to assess (1) responses to specific compounds or groups of compounds or (2) general responses in the absence of direct information on the factors in the environment that are causal for the responses. Figures 4 and 5 show the elements in a program for health monitoring that includes these two types of ESBs. The activities include industrial hygiene type evaluations, biomonitoring on personnel, programs for health surveillance. Combined with the exposure monitoring results, ESBs related to specific compound-related responses provide the basis for real-time and more long-term risk assessments.

These integrative ESBs can also provide positive responses in the absence of information on the specific causative factors. These more generic responses, indicating a problem in the healthfulness of the environment, provide a greater challenge for risk assessment and an obligation for consideration of follow-up to identify and assess the consequences of these ESB responses for personnel. The set of auxiliary studies (Figure 5) might include further characterization of the atmospheric contaminants, specific toxicity studies with the ESB system itself and with intact animals, and plans for prospective epidemiologic studies on select personnel. These activities would permit assessment of the potential long-term consequences of these exposures. In relation to short-term risk assessment, interpretation of the generic, integrative responses is problematic and may be difficult to weigh in relation to other mission requirements.

State-of-the-Art - 2001

Cell and tissue ESBs represent a broad array of possible systems for evaluating contaminants and generic responses to contaminants in the deployed environment. The diversity of possibilities results from advances in many areas, primarily in biotechnology, cell culture methods, and in tissue replacement/regeneration technologies. With individual cells, it is possible to engineer specific groups of genes with promoters that can respond to a variety of chemical probes. Many cell systems could be designed to serve as markers for generic toxicity by responding to stimuli with measurable responses including release of enzymes or production of colored/fluorescent products after promoter activation. These systems are much more easily developed from transformed cells because of the difficulty of maintaining primary cultures of specific cells in culture. A tissue based system, with cells from

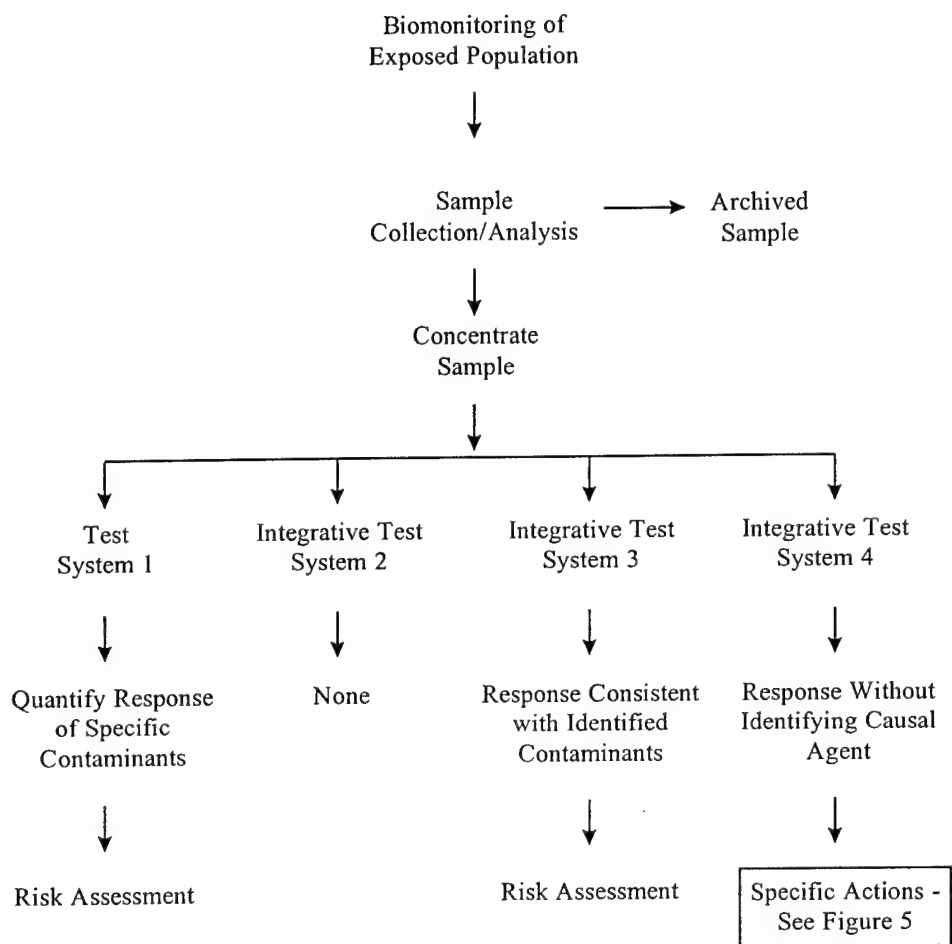


Figure 4. Overall Plan for Environmental Monitoring in Deployed Environments

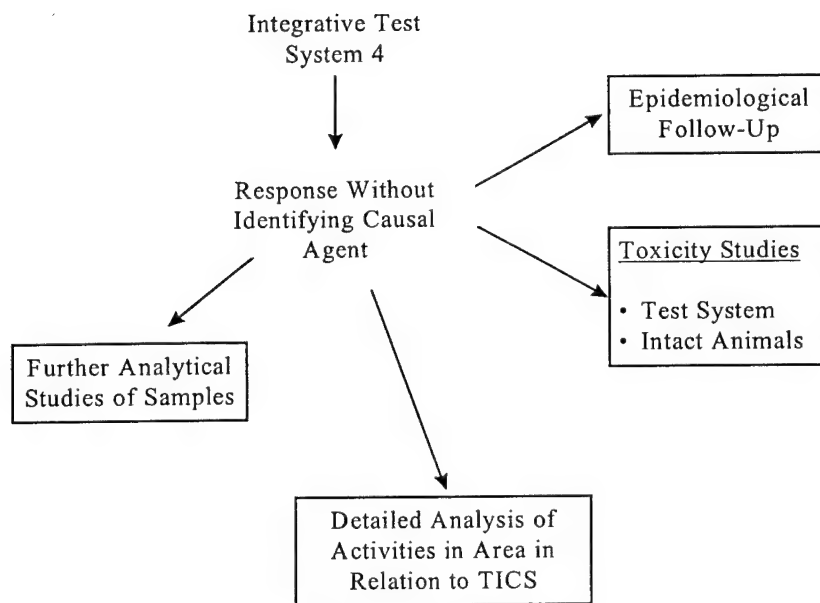


Figure 5. Plan of Steps Required When Integrative Response Systems Register Positives without Identification of Causative Agents in Air/Water Samples

specific organs, could respond to stimuli and provide a generic tool to assess tissue/organ function. For instance, liver cells grown on slides with collagen can reproduce activation of various constituents to assess organ system toxicity. Other cell based systems, with heart or neuronal cells on a chip, could serve to assess integrated function in cell aggregates. The cell systems need to be coupled to monitoring strategies that allow ease of identification of positive results – such as changes in color, fluorescence, altered electrical characteristics of excitable cells, conductivity of a washing fluid or alteration in absorption/fluorescence in the cells themselves.

Other areas of rapid development are in genomics and proteomics. The responses of ESBs can range from alterations in regulation/expression of single genes by specific molecules to control of multiple genes by regulation of cell batteries that are associated with particular cellular functions. To measure these latter responses would require genomic tools to assess the status of mRNA derived from many genes and the knowledge base that ascribes these gene changes to alterations in specific functional behaviors of the cell. These behaviors include oxidative stress responses, control of DNA repair, and control of cell proliferation/cell death (apoptosis). In addition to genomic changes, state-of-the-art methodologies can also assess protein level modifications in cells, including changes in protein concentrations by 2-dimensional protein separation and more recently evaluation of changes in protein processing within cells following exposure to xenobiotics. Analysis of cells (or even protein solutions) could identify adducted proteins and potentially identify reactive compounds (such as organophosphate nerve agents) in the environment.

In the arena of cell and tissue based ESBs, the technology available is astonishing. However, much of the available methodologies are still laboratory level research modules and require validation and experience in their use to make them attractive ESBs and fashion them for use in remote operations. Careful articulation of design criteria for these cell and tissue ESBs would provide incentive for development of these systems in various research laboratories.

While the state-of-the-art in cell and tissue ESBs may only be limited by the imagination and cleverness of molecular biologists, the second question that needs to be asked is what types of systems are most needed for current deployed situations. Perhaps the intersection of ESB needs with the capabilities available for engineering cellular systems from molecular biology will strongly influence design of the initial suite of integrative ESBs. A series of questions might be asked about the toxicological characteristics of the environment. These questions would be directed at responses of specific organ systems. The main tissues of interest historically have been the pulmonary system, nervous system, and immune system. In addition, it would be advantageous to test to see if the environment contained compounds that were directly reactive with macromolecules or were activated to form toxicity. These interests could define the types of cell and tissue ESBs that should be considered for initial development.

Some critical issues for integrative ESB systems include:

- Sample preparation. For instance, what is the pH of solutions made by adding water to atmospheric concentrates?
- Contact site toxicity of environmental compounds. For instance, are these atmospheres toxic to pulmonary epithelial cells during gas phase exposures of the cell cultures?
- General reactivity of the environmental compounds. For instance, are protein/DNA adducts formed in cell systems during exposure to the atmospheric components?

- Metabolic activation to toxic contaminants. For instance, are there compounds present that appear to be activated to produce toxic responses in liver cells?
- Direct action on integrated cell functions. For instance, does exposure of nervous system cell lines or synchronously beating cardiocytes show evidence of alterations in functions?

Enumerating a list of critical questions of this kind could help structure the thinking about the scope of integrative cell and tissue ESBs that might be developed initially. A second point to raise in this list of ESBs is in relation to the types of studies that could be done "real-time" to assess the environment and how these same ESBs might become the system for use to provide more in-depth characteristics of toxicity from atmospheric contaminants.

Technical Challenges

Several major challenges, both programmatic and technical, can be identified. Programmatic issues include:

- Should the Army try to identify a best suite of preferred capabilities for assessing "healthfulness of the environment" or have they pursued a more narrow focus on specific response, as suggested above, for more limited sets of endpoints?
- Will the Army focus solely on full remote, continuous operation? This type of remote laboratory has not been pursued in the past; thus posing new technology needs for field implementation of cellular and tissue based ESBs.
- What kind of remote laboratory or intermittently manned laboratory will be established to allow cell and molecular approaches to ESBs to be automated?

Technical issues include the following.

- How will these integrative ESB positive results be interpreted for human health risk assessment? In the absence of knowledge of the presence of specific causative toxicants in an environment, the risks to deployed forces from the atmosphere will be difficult or impossible to predict based on the responses of ESBs alone.
- How will positive responses from ESBs serve as flags for more extensive field evaluation of atmospheric components, estimation of deployed area work practices, toxicity testing and prospective epidemiology?
- How will more integrative cellular responses at the genomic or proteomic level be examined and included in ESB programs and associated risk assessments for these deployed environments?

Long-Term Possibilities

The longer-term outlook, even for automation of very sophisticated assays with genomic or proteomic, evaluation of responses is very good. The human genome and proteome projects have made rapid

progress due mainly to improvements in robotics and high throughput technologies. These approaches could potentially serve as a blueprint to move to more elaborate ESBs based on these commercialized technologies. The use of integrative responses in gene control and in measuring proteins altered after exposure appear to be very promising future areas to develop integrative ESBs. In addition, a long-term strategy to simultaneously develop ESBs and a sufficient toxicity/response data on libraries of compounds could provide a more rational selection criterion for these ESBs.

The development of data on responses of the ESBs to known compounds could also serve to give rules for how these responses might be applied to assist in short and long-term human health risk assessments. Another technology to watch is artificial organ development where techniques to seed normal cells onto matrices have been partially successful in developing aggregates of cells with organ level-integrated responses. Techniques developed allowing long-term viability of primary cells in culture should be carefully monitored for potential use with remote ESBs.

Summary

Cell and tissue based ESBs can be constructed from normal or bioengineered organisms. These ESBs can give information on single or multiple chemical exposures and would be most easily used for risk assessments when the constituents of the atmosphere are fairly well documented. The need to provide remote, continuous reading instrumentation for use with cells and/or tissue based ESBs appears to be a formidable challenge today. Some ideas for cell and tissue ESBs are discussed in relation to either broad surveys of the healthfulness of the environment or in relation to toxicity/risk assessments for particular components of the atmosphere. Though likely to be beset by growing pains, the longer-term prospects for these automated cell ESBs are extremely good.

Whole Organism Biosensors by Dr. William T. Waller, University of North Texas

Introduction

Aquatic toxicologists are guided by three principles:

- you only find what you are looking for
- it's the exposure stupid
- only living material can measure toxicity

Often when you approach a municipality in Texas where the municipality's water quality meets certain standards, you are greeted by a sign containing the words "Superior Public Water System, The State of Texas".

What does the sign convey to the public and what does it really mean? The public generally believes, based on informal surveys, that the sign indicates the water in the municipality is safe to drink and in some sense that is correct. However, what it really means is that the water does not exceed the limits set for a series of prescribed parameters. It does not say that there can't be chemicals that are not measured that could cause problems nor does it say that there can't be combinations of chemicals interacting in ways that could cause problems. You only find what you are looking for, and you only find it if the methods you are using are sufficiently sensitive to detect the chemical's presence. The real biosensor in this case is the public.

Exposure may be defined as the magnitude, duration, and frequency with which an organism interacts with biologically available toxicants. This principle forms the basis for the evaluation of toxicants. All acute and chronic toxicity determinations examine the relationship between these parameters as part of the quest for "biologically safe concentrations". A chemical that is not biologically available may be measured using analytical methods but its impact on aquatic organisms may be overestimated based on those results. Water quality standards may take the form of the example that follows based on copper. The "Gold Book" (USEPA, 1986) states, "The procedures described in the Guidelines for Deriving Numerical Water Quality Criteria for the Protection of Aquatic Organisms and Uses indicate that, except possibly where a locally important species is very sensitive, freshwater aquatic organisms and their uses should not be affected unacceptably if the 4-day average concentration (in ug/L) of copper does not exceed the numerical value given by $e^{(0.8545[\ln(\text{hardness})]-1.465)}$ more than once every 3 yr on the average and if the 1-hour average concentration (in ug/L) does not exceed the numerical value given by $e^{(0.9422[\ln(\text{hardness})]-1.464)}$ more than once every 3 yr on average. For example, at hardnesses of 50, 100, and 200 mg/L as CaCO₃ the 4-day average concentrations of copper are 6.5, 12, and 21 ug/L, respectively, and the 1-hour average concentrations are 9.2, 18, and 34 ug/L." As these statements indicate there is a magnitude, duration, and frequency component incorporated into the statement. The knowledge that copper toxicity is altered by the hardness of the water also allows the incorporation of a biological availability component. Nonetheless, the standard is for the toxicant copper in the absence of all other toxicants. It is unlikely that those conditions ever exist in nature. While we understand exposure, our understanding of mixtures of toxicants is poor at best.

The following quote comes from a 1990 publication by John Cairns, Jr. and Don Mount:

"No instrument has yet been devised that can measure toxicity! Chemical concentrations can be measured with an instrument but only living material can be used to measure toxicity."

Living material, integrates the totality of its environment, deals with mixtures, with things that are not measured, and reacts to biologically available toxicants. Whole organisms are the ultimate integrators. The current Whole Effluent Toxicity (WET) program that is part of the National Pollutant Discharge Elimination System permitting recognizes the integrating properties of living organisms and monitoring effluents with organisms is part of almost all permits to discharge issued in the US. Therefore, whole organism biosensors make sense in a toxicological context. However, even if they work perfectly, seldom do they tell us what chemical or mixture is causing the problem, only that a problem is occurring. Chemical methods, like the Toxicity Identification Evaluation methods, are needed to identify the toxicants.

Whole organism biosensors have been around for a long time. Tasters were routinely employed by royal families, and canaries were used by miners as a check against toxic gases. Biological surveys have been used to monitor for pollution for 100+ years. The use of organisms to monitor for adverse water quality conditions, in this sense, is almost always a post hoc process used to establish current "health". In cases where there is a single discharge, it has sometimes been possible to use survey methods to measure conditions above and below an outfall and to draw inferences about the causes of the observed impact.

The purpose of a real-time or time relevant biomonitoring system is to provide a means of continuous water quality monitoring with realistic exposures and to associate that exposure with parcels of water that cause an abnormal response on the part of the biosensor. Henderson and Pickering (1963), Jackson and Brungs (1966), and Hasselrot (1975) deployed fish in flowing waters where they were

periodically examined for signs of mortality or stress. While these employments worked for lethal conditions, they were not continuous, and their real-time or time relevancy was dependent on the frequency with which the fish were examined.

Some 30 yr ago, Cairns (1967) and Cairns et al. (1970 a,c) proposed the implementation of a continuous real-time monitoring system for deployment in a watershed to monitor and manage these water resources. Some of the problems in the early days of real-time whole organism biomonitors were related to handling the volume of data generated and the status of electronics at that time. As the availability of computers and computing power increased and as solid state electronics became available these problems lessened.

As real-time whole organism biomonitors have evolved, two broad paths have been taken in their deployment. In one case, the monitors have been deployed at sites where they are exposed to waters that are either pumped to the organisms or they are deployed directly in the aquatic system being monitored but in close proximity to the facility where the data are being managed and analyzed. In these instances the monitors are often hardwired to the facility.

In the second path, the biomonitors are strategically deployed in a watershed at sites that may be some distance from any facility. In this deployment the system often uses electricity generated with solar cells and communication between the site and a central management facility is accomplished using satellites or digital modems.

Biosensors

The biosensors used today in real-time whole organism systems cover a broad spectrum of biological organisms and physiological/behavioral endpoints (changes in activity, ventilation, heart rate, coughing rate, oxygen consumption, bivalve gape, fluorescence, etc.). Fish have been widely used as biosensors in these systems. Dr. John Cairns, Jr. was one of the earliest proponents of these systems and his influence can be seen in the list of references at the end of the paper for which he is an author or co-author with a sequence of students who have contributed to this field (Sparks, Waller, Morgan, Gruber, van der Schalie, Westlake, and Doane). Many others have used fish as real-time biosensors (Geller, 1984; Byrne, 1971; Camougis, 1960; Geller, 1984; Gerhardt, 1994; O'Hara, 1971; Roberts, 1973; Shamburg et al. 1967; Slooff, 1979; Spoor et al, 1971; Koeman et al, 1978; and Evans and Wallwork, 1996). Bivalves have also been used as real-time biosensors (Allen et al, 1996; Allen et al, 2001; Borcharding, 1992; De Zwart et al, 1995; Ham and Peterson, 1994; Kees et al, 1989; Kramer et al, 1989; Sloof et al, 1983; Englund et al, 1994; Jenner et al, 1989; Borcharding and Volpers, 1994). Other organisms that have been used include insects (Gerhardt et al, 1994; Gerhardt, 1996; Morgan et al, 1984; Morgan et al, 1987a,b); protozoans (Tahedi and Hader, 1999); decapods (Idoniboye-obu, 1997) and amphipods, Cladocera, bacteria, algae and tadpoles (Campanella et al, 2000; Michels et al, 1999; Caspers, 1988; Gerhardt, 1995, 1996, Willingham and Anderson, 1996; Willingham and Anderson, 1967; Stoks, 1998).

Relationship to Human Health

The relationship between whole organism biosensors and human health effect(s) has not been directly established. In Europe where whole organism biosensors have been deployed for source water protection, biosensors are used as alarms to warn of the presence of factors that need to be investigated by other means, before the water is used for human consumption. When the alarm sounds, the

causative agent is generally not known, but steps are taken to prevent the water from being used until either the agent is identified, and the risk deemed acceptable, or the problem has passed.

In principle it makes sense that whatever the deployed systems look like they should detect the presence of TIC/TIMs, CWA/BWAs at concentration levels below those that will have an impact on the deployed forces and/or with sufficient time relevance that the impact is minimized. The goal is not to have the troops (which are whole organism biosensors) be the first to detect a problem. Whole organism biosensors that are currently available and those that might be developed have the potential to provide this early warning although additional testing is needed to combine the best biological sensors for this particular deployment, and to integrate them into a single system.

Systems

A variety of real-time biomonitoring systems have been developed and deployed. In Europe more than 45 sites on the Elbe, Rhine and tributaries thereof are being continuously monitored using some combination of fish, mussels, cladocerans, algae, and bacteria. Almost all the units deployed at these sites are commercially available. Only a fraction of this number of sites are currently being monitored in the US and these are generally, but not exclusively, research monitors that are not commercially available. However, some of these monitors have been constructed from off-the-shelf hardware and are not proprietary (Allen et al, 2001). In Europe some of the sites are being monitored with multiple species. However, in most cases these multi-species monitors are not integrated single systems but rather individual systems deployed at the same site. In Europe many of these monitoring systems are used as part of a drinking source water protection scheme.

Some of the commercially available systems include:

- LimCo International offers a multi-species Biological Early Warning System (BEWS) capable of measuring 96 chambers with a sampling frequency of 20 Hz. Organisms include, Plecoptera (locomotion and ventilation), Ephemeroptera (locomotion), Chironomidae (locomotion), and *Rana temporaria* (locomotion).
- The Mosselmonitor® (Delta Consult, Kapelle, The Netherlands) is a biomonitor that uses the valve position and the activity of eight *Dreissena polymorpha* (freshwater), *Mytilus edulis* (saltwater) to monitor water quality. This device has been used to monitor the intake of a drinking water plant on a river system, for continuous monitoring of the rivers Rhine and Meuse in combination with other biological early warning systems, for monitoring industrial effluents in a tidal estuary, and for control of cooling water chlorination for antifouling purposes.
- Biological Monitoring, Inc. Blacksburg, Va. offers a BIO-SENSOR® Automated and Continuous Water Quality Monitoring system. The Bio-Sensor® is a BEWS which utilizes fish, electronics, and computer technology. This water quality monitoring system operates automatically and on a real-time basis. The technology relies upon the fact that all fish generate a microvolt level bioelectric field, the result of their neuromuscular activities. Non-contact submerged electrodes within each monitoring chamber receive these signals.
- NANCIE has a fish monitoring system called GYMNOTOX®: Biological detector of polluted water based on the electric discharges delivered by tropical fish. Gymnotox®

exploits the electric signals naturally emitted by fish originating in fresh waters of South America, *Apteronotus albifrons*. Its endpoint is based on measurements of the frequency of the electric discharges of fish.

Another monitor from this group exploits the photosynthetic activity of algae (*Scenedesmus subspicatus*) by measuring emitted fluorescence.

- A commercial BEWS based on the phototactic behavior of *Daphnia magna* is also available (van Hoof et al, 1994).

Integration

It is well known that not all species respond to toxicants in the same manner nor do they all possess the same level of sensitivity. Therefore, it is important that multiple species be used in any widespread monitoring effort (Cairns and van der Schalie, 1980, Brown, 1976, Price, 1978). One of the goals for military deployment should be integration of the most applicable BEWS into a single control system.

Strengths and Weaknesses

- Strengths

The strengths of the BEWS have been discussed throughout the paper and are best summarized by the Cairns and Mount quote given earlier in this White Paper.

- Weaknesses

In terms of the normal application of BEWS there are areas for which these sensors do not provide time relevant information. These include factors such as bioconcentration and biomagnification (the tissues of the bivalves can be sampled for analysis in bioaccumulation studies), nutrient and organic enrichment (stimulation), and genotoxic responses (Waller et al, 1996). While not a weakness peculiar to BEWS, the question, 'What does it mean?' is always pondered. If mussels close in response to a toxicant in the environment, what does that mean to the higher levels of ecological organization (population, community, ecosystem) and, of greatest concern, to human health. The importance of this question is related to the intent of the monitor. If the monitor is a general warning device of some adverse conditions or even a change in conditions, then the question is less relevant. If the monitor is to provide information on the likely degree and nature of "harm," the question is much more relevant.

The systems discussed to this point are all based on the single medium, water. Airborne contaminants are not addressed by these systems. However, it may be possible to incorporate some aspects of airborne toxicants into these systems by continuously saturating water with air from a site and then use a BEWS to monitor that water. However, there are biological monitors that are designed specifically for airborne contaminants. An example of these can be found in the work of Bromenshenk (1995, 1993, 1992, and 1985). Bromenshenk has developed a biosensing system for airborne contaminants based on the honey bee. Data from studies by Bromenshenk and colleagues indicate that by placing hives on a site and measuring chemicals brought back to the hive, heavy metals, radionuclides, organic solvents pesticides and explosives can be detected. According to information presented on the web site,

the researchers believe that bees may also be useful for detecting microbes in the environment (e.g. microbial pesticides and biowarfare agents) and possibly land mines.

Future

There must be certain criteria set for evaluating BEWS for each particular application. Published criteria (de Zwart et al, 1995; Koeman et al, 1978; Cairns, 1979; Cairns and van der Schalie, 1980, Diamond et al, 1988) include:

- flow-through or *in situ* exposure of organisms;
- (semi)continuous measurements of behavioral or physiological patterns in organisms;
- automated detection of (short-term) changes in environmental conditions by evaluating the reaction of the organisms under observation;
- fast response;
- high sensitivity for a broad spectrum of pollutants
- low incidence of false alarm indication;
- test organisms should preferably be easily obtainable, manageable in size, comparatively long-lived, hardy enough to be handled, and responding to a wide range of stress intensity;
- ease of operation;
- low maintenance;
- flexible design for application in the laboratory as well as under field conditions
- comprehensible and fully documented output; and
- availability of elaborate documentation (operational manual, technical manual, trouble-shooting guidance, full description of evaluation of methodology, statements on compound specific detection limits, etc.)

Dr. Waller suggests two levels of deployment for BEWS in military applications. The first level would be deployment in which the biosensors would be maintained in a portable lab that could pull up beside a water source, deploy a hose, and start pumping water into the lab and through the sensor housing. Multiple organisms could be exposed to the same water stream. The data collected could be analyzed on the spot and alarms sounded in the event of the presence of toxic material and/or the data could be transmitted by satellite to a remote center for risk assessment and management. A mobile lab with some of these characteristics was constructed by Morgan et al. (1988).

The second level of deployment might employ a network of biosensors covering a large geographical area. In this case the same type biosensors, or some subset thereof, might be deployed directly into the aquatic environment throughout a watershed to monitor the quality of the water at that scale. In this example the data from the remote sites might be transmitted via satellites to a central command center where risk assessment and management decisions could be made.

The near-term (5 yr) future of BEWS, as it relates to potential military application, lies in two areas: 1) evaluating existing BEWS in side by side comparisons (USEPA is in the process of comparing a fish monitor, a clam monitor, an algal monitor and a *Daphnia* monitor) to determine the breadth of contaminants that each sensor responds to and their relative sensitivities to the groups of chemicals deemed appropriate for this particular application. Once this is accomplished, 2) the most promising of the existing technologies that meet the criteria listed above should be combined into a single platform that is then evaluated against the criteria.

The future of real-time whole organism biosensors in the longer term will look like that given in Figure 6. This sensor would be ideally suited for many applications including military. Assuming that the site that is to be monitored allows for the survival of the biosensor, the information gathered would be invaluable for risk assessment. However, the development of this sensor is not going to happen in a vacuum. It is important that the nanntechnologists and the scientists involved in whole organism, tissue, cellular and molecular research work together to develop and produce this sensor.

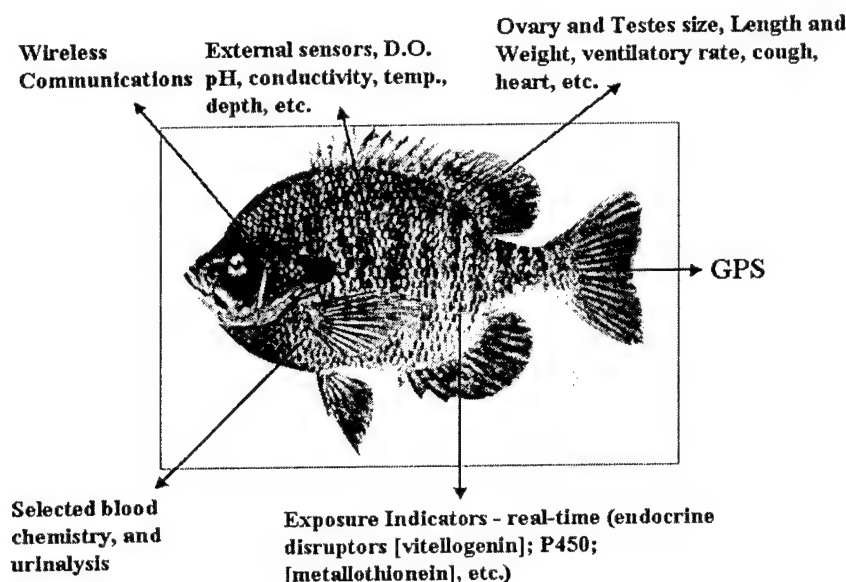


Figure 6. Future Real-Time Whole Organism Biosensors

References

- Allen H.J., W.T. Waller, M.F. Acevedo, E.L. Morgan, K.L. Dickson, and J.H. Kennedy. 1996. A minimally-invasive technique to monitor valve-movement behavior in bivalves. *Environ Technol* 17:501-507.
- Allen, H.J., W.T. Waller, J.H. Kennedy, K.L. Dickson, M.F. Acevedo, and L.P. Ammann. 2001. Real-time whole organism biomonitoring - Deployment, status, and future. AWRA. Annual Spring Speciality Conference Proceedings. Warwick, J.J. [Ed] *Water Quality Monitoring and Modeling*. AWRA., Middleburg, VA. TPS-01-1, 284 pp.
- Borcherding, J., 1992. Another early warning system for the detection of toxic discharges in the aquatic environment based on the movements of the freshwater mussel *Dreissena polymorpha*, *Limnologica Aktuell* Vol. 4, Neumann/Jenner eds. The Zebra Mussel *Dreissena polymorpha*, Gustav Fischer Verlag, Stuttgart.
- Borcherding, J., and M. Volpers. 1994. The "Dreissena-monitor" first results on the application of this biological early warning system in the continuous monitoring of water quality. *Water Sci. Technol.* 29:199-202.

- Bromenshenk, J.J., G.C. Smith, and V.J. Watson. 1995. Assessing Ecological Risks in Terrestrial Systems with Honey Bees. In: *Biomonitoring and Biomarkers as Indicators of Environmental Change*, F.M. Butterworth, ed. Plenum Press, New York. Chapter 2:9-30.
- Bromenshenk, J.J. and G. DeGrandi-Hoffman. 1993. PC BEEPOP, a Microcomputer Model of the Population Dynamics and Economics of Honey Bee Colonies. Feature Article in *American Entomologist* 39: 231-237.
- Bromenshenk, J.J. 1992. Site Specific and Regional Monitoring with Honeybees: Case Study Comparisons. In: *Ecological Indicators*, D.H. McKenzie, D.E. Hyatt, V.J. McDonald, eds. Elsevier Applied Science, London and New York. Chapter 39:689-704.
- Bromenshenk, J.J., S.R. Carlson, J.C. Simpson, J.M. Thomas. 1985. Pollution Monitoring in Puget Sound with Honey Bees. *Science* 227: 632-634.
- Brown, V.M. 1976. Advances in testing the toxicity of substances to fish. *Chem. Ind.* 4:143-149.
- Byrne, J.E. 1971. A further contribution to using ultrasonic sensors for fish activity studies. *Trans. Am. Fish. Soc.* 100:792-794.
- Cairns, J. Jr. 1967. The use of quality control techniques in the management of aquatic ecosystems. *Wat. Res. Bull.* 3(4):47-53.
- Cairns, J. Jr., R.E. Sparks, and W.T. Waller. 1970a. Biological systems as pollution monitors. *Res. and Development*:22-24.
- Cairns, J. Jr., K.L. Dickson, R.E. Sparks, W.T. Waller. 1970c. A preliminary report on rapid biological information systems for water pollution control. *J. Wat. Pollut. Control Fed.* 42:685-703.
- Cairns, J. Jr. 1979. Biological Monitoring-Concept and Scope. pp. 3-20. In J. Cairns, G.P. Patil, and W.E. Water (eds.) *Environmental Biomonitoring, Assessment, Prediction and Management-Certain Case Studies and Related Quantitative Issues*. International Co-operative Publishing House, Fairland, MD, USA.
- Cairns, J. Jr., and W.H. van der Schalie. 1980. Biological monitoring. Part 1. Early warning systems. *Wat. Res.* 14:1179-1196.
- Cairns, J. Jr., and D.I. Mount. 1990. Aquatic Toxicology. *ES&T* 24(2):154-161.
- Camougis, G. 1960. Recording bioelectric action potentials from aquatic animals. *Turtox News*. 38:156-167.
- Campanella, L., F. Cubada, M.P. Sammartino, and A. Saoncella. 2000. An algal biosensor for the monitoring of water toxicity in estuarine environments. *Wat. Res.* 35(1):69-76.
- Caspers, N. 1988. Kritische Betrachtung des "Dynamischen Daphnientests". *Z. Wasser-Abwasser-Forsch.* 21:152-154.

De Zwart, D., K.J.M. Kramer, and H.A. Jenner. 1995. Practical experiences with the biological early warning system, "Mosselmonitor". *Env. Tox. and Water Qual.* 10:237-247.

Diamond, J., M. Collins, and D. Gruber. 1988. An overview of automated biomonitoring-past developments and future needs. p 23-39. In D.S. Gruber, and J.M. Diamond (eds.) *Automated Biomonitoring: Living Sensors as Environmental Monitors*. Ellis Horwood, Chichester, United Kingdom (UK).

Englund, V.P., M.P. Heino and G. Melas. 1994. Field method for monitoring valve movements of bivalved molluscs. Technical note. *Water Res.* 28:2219-2221.

Evans, G. and J. Wallwork. 1996. The WRC fish monitor and other biomonitoring methods. In D.S. Gruber and J.M. Diamond, eds., *Automated Biomonitoring*, Ellis Horwood, Chichester, UK. 75-90.

Geller, W. 1984. A toxicity warning monitor using the weakly electric fish *Gnathonemus petersi*. *Wat. Res.* 18:1285-1290.

Gerhardt, A., E. Svensson, M. Clostermann, and B. Fridlund. 1994. Monitoring of behavioural patterns of aquatic organisms with an impedance conversion technique. *Environmental International* 20(2):209-219.

Gerhardt, A. 1995. Monitoring behavioral responses to metals in *Gammarus pulex* (L.) with impedance conversion. *Environ. Sci. and Pollut. Res.* 2(1):15-23.

Gerhardt, A. 1996. Behavioral early warning responses to polluted water. Performance of *Gammarus pulex* L. (Crustacea) and *Hydropsyche angustipennis* (Curtis) (Insects) to a complex industrial effluent. *Environ. Sci. and Pollut. Res.* 3(2):63-70.

Ham, K.D., and M.J. Peterson. 1994. Effect of fluctuating low-level chlorine concentrations on valve-movement behavior of the Asiatic clam (*Corbicula fluminea*), *Env. Tox. Chem.* 13(3):493-498.

Hasselrot, T.B. 1975. Bioassay methods of the National Swedish Environmental Protection Board. *J. Wat. Pollut. Control Fed.* 47:851-857.

Henderson, C. and Q.H. Pickering. 1963. Use of fish in the detection of contaminants in water supplies. *J. AM. Wat. Wks. Ass.* 55:717-720.

Hynes, H.B.N. 1966. *The biology of polluted waters*. Liverpool University Press.

Idoniboye-obu, B. 1977. Recording bioelectric potentials of marine decapod Crustacea by remote electrodes: A procedure for monitoring hydrocarbon pollution. *Environ. Pollut.* 12:159-166.

Jackson, H.W. and W.A. Brungs, Jr. 1966. Biomonitoring industrial effluents. *Ind. Waste Eng.* 45:14-18.

Jenner, H.A., F. Noppert, and T. Sikking. 1989. A new system for the detection of valve movement response of bivalves. *KEMA Sci. Tech. Rep.* 7:91-98.

- Kees, J.M., H.A. Kramer, H.A. Jenner, and D. de Zwart. 1989. The valve movement response of mussels: A tool in biological monitoring. *Hydrobiologia*. 188/189:443.
- Keup, L.E., W.M. Ingram, K.M. Mackenthum. 1967. *Biology of Water Pollution*, A collection of selected papers on stream pollution, waste water, and water treatment. U.S. Dept. Interior, Fed. Water Poll. Ctrl. Admin. Cincinnati, Oh. 290 p.
- Koeman, J.H., C.L.M. Poels, and W. Sloff. 1978. Continuous biomonitoring systems for the detection of toxic levels of water pollutants, P. 339-347. In O. Hutzinger, L.H. Van Lelyveld, and B.C.J. Zoeteman (eds.) *Aquatic Pollutants, Transformations and Biological Effects*. Pergamon Press, Oxford, UK.
- Kolkwitz, R. and M. Marsson. 1908. Oekologie der pflanzlichen Saprobien, Berichte der Deutschen Botanischen Gesellschaft, 26a:505-519.
- Kramer, K.J.M., H.A. Jenner, and D. de Zwart. 1989. The valve movement response of mussels: a tool in biological monitoring. *Hydrobiol.* 188/189:433-443.
- McDonald, M. 1885. Effect of waste products from Page's Ammoniacal Works upon young shad fry. Bull. U.S. Fish. Com., Washington, 5:311-314.
- Michels, E., M. Leynen, C. Cousyn, L. De Meester, and F. Ollevier. 1999. Phototactic behavior of *Daphnia* as a tool in the continuous monitoring of water quality: Experiments with a positively phototactic *Daphnia magna* clone. *Wat. Res.* 33(2):401-408.
- Morgan, E.L., R.C. Young, and C. Crane. 1984. Automated multi-species biomonitoring employing fish and aquatic invertebrates of various trophic levels. in *Freshwater Biological Monitoring*, D. Pascoe and B.W. Edwards, Eds. Pergamon Press, Oxford, UK pp 75-78.
- Morgan, E.L., R.C. Young, M.D. Smith, and K.W. Eagleson. 1987a. Rapid toxicity detection in water quality control utilizing automated multi-species biomonitoring for permanent space stations, *J. Env. Sci.* 30(2):47-49.
- Morgan, E.L., R.C. Young, M.D. Smith, K.W. Eagleson. 1987b. Proposed application of automated biomonitoring for rapid detection of toxic substances in water supplies of permanent space stations. *J. Env. Sci.* 47-49.
- Morgan, E.L., R.C. Young, and J.R. Wright, Jr. 1988. Developing portable computer-automated biomonitoring for a regional water quality surveillance network. In *Automated Biomonitoring*, [eds] D. Gruber and J. Diamond. Ellis Harwood, Ltd., UK pp. 127-141.
- O'Hara, 1971. A continuously monitored respiration chamber for fish. *Wat. Res.* 5:143-145.
- Price, D.R.H. 1978. Fish as indicators of water quality. *J. Wat. Poll. Cntrl. Fed.* 285-296.
- Roberts, M.G., D.E. Wright, and G.E. Savage. 1973. A technique for obtaining the electrocardiogram of fish. *Comp. Biochem. Physiol.* Vol. 44A:665-668.
- Shamburg, F.D., T.E. Howard, and C.C. Walden. 1967. A method to evaluate the effects of water pollutants on fish respiration. *Water Res.* 1:731-737.

Slooff, W. 1979. Detection limits of biological monitoring systems based on fish respiration. *Bull. Env. Contam. Tox.* 23:517-523.

Slooff, W., D. de Zwart, and J.M. Marquenie. 1983. Detection limits of a biological monitoring system for chemical water pollution based on mussel activity. *Bull. Env. Contam. Toxicol.* 30:400-405.

Spoor, W.A., W.T. Neiheisel, and R.A. Drummond. 1971. An electrode chamber for recording respiratory and other movements of free-swimming animals. *Trans. Am. Fish. Soc.* 100:22-28.

Stoks, P.G. 1998. From river water to drinking water: online systems in water quality control. IWSA Workshop, Online Monitoring. Amsterdam. 6 pp.

Tahedl, H. and D.P. Hader. 1999. Fast examination of water quality using the automatic biotest ECOTOX based on the movement behavior of a freshwater flagellate. *Wat. Res.* 33:426-432.

Tarzwel, C.M. 1965. *Biological problems in water pollution, Third seminar 1962*, U.S. Dept. of Health, Education, and Welfare, Public Health Service, Division of Water Supply and Pollution Control, Cincinnati, Oh. 424 p.

USEPA. 1986. Water Quality Criteria for Water, 1986. EPA 440/5-86-001. Office of Water, Regulations and Standards, Washington, DC.

USEPA. 1988. Methods for aquatic toxicity identification evaluations: Phase I toxicity characterization procedures. EPA-600/3-88-035. Environmental Research Laboratory, Duluth, MN.

USEPA. 1989a. Methods for aquatic toxicity identification evaluations: Phase II toxicity identification procedures. EPA-600/3-88-036. Environmental Research Laboratory, Duluth, MN.

USEPA. 1989b. Methods for aquatic toxicity identification evaluations: Phase III toxicity confirmation procedures. EPA-600/3-88-037. Environmental Research Laboratory, Duluth, MN.

USEPA. 1991a. Methods for aquatic toxicity identification evaluations: Phase I toxicity identification procedures. Second Edition. EPA-600/6-91-003. Environmental Research Laboratory, Duluth, MN.

USEPA. 1991b. Sediment toxicity identification evaluation: Phase I (characterization), Phase II (identification) and Phase III (confirmation) modifications of effluent procedures. EPA-600/6-91-007. Environmental Research Laboratory, Duluth, MN.

USEPA. 1992. Toxicity identification evaluation: characterization of chronically toxic effluents, Phase I. EPA-600/6-91-005. Environmental Research Laboratory, Duluth, MN.

USEPA. 1993a. Methods for aquatic toxicity identification evaluations: Phase II toxicity identification procedures for samples exhibiting acute and chronic toxicity. EPA-600/R-92-080. Environmental Research Laboratory, Duluth, MN.

USEPA. 1993a. Methods for aquatic toxicity identification evaluations: Phase III toxicity confirmation procedures for samples exhibiting acute and chronic toxicity. EPA-600/R-92-081. Environmental Research Laboratory, Duluth, MN.

van Hoof, F., H. Slutys, J. Paulussen, D. Berckmans and H. Bloemen. 1994. Evaluation of a biomonitor based on the phototactic behaviour of *Daphnia magna* using infrared detection and digital image processing. *Wat. Sci. Tech.* 30:79-86.

Waller, W.T., L.P. Ammann, W.J. Birge, K.L. Dickson, P.B. Dorn, N.E. LeBlanc, D.I. Mount, B.R. Parkhurst, H.R. Preston, S.C. Schimmel, A. Spacie, G.B. Thursby. 1996. Session 6, Predicting instream effects from WET tests, Discussion Synopsis. In: Whole Effluent Toxicity Testing: An Evaluation of Methods and Prediction of Receiving System Impacts. D.R. Grothe, K.L. Dickson, and D.K. Reed-Judkins, Eds. Society of Environmental Toxicology and Chemistry, Pensacola, Fl. 346 p.

Willingham, C.A. and K.J. Anderson. 1966. Use of microorganisms for detecting toxicants in water. Part I. *Wat. Sew. Wks.* 113:464-467.

Willingham, C.A. and K.J. Anderson. 1967. Use of microorganisms for detecting toxicants in water. Part I. *Wat. Sew. Wks.* 114:25-28.

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I. Introduction

The detection of TICs and TIMs in the environment is a daunting task considering the number of materials that must be detected. This study focuses on the measurement of a broad spectrum of these substances in air and water samples. Furthermore, the emphasis is placed on technologies that provide a fieldable capability, some in the near term but others that will take longer to develop. Paramount in this study is the realization that the variation in natural and man-made chemical and biological background in the area of deployment may be quite high and must be strongly considered.

II. Generic Detection

Given the large number of materials that must be investigated, the detection methodologies employed must be sensitive to the physical and molecular properties of the substances in order to make an accurate identification. Furthermore, in most cases, techniques must be employed that separate the chemicals of interest from each other and from the background. Important properties that distinguish between different members of the TIC/TIM class include: vapor pressure, molecular weight, molecular structure, polarity, hydrophobicity, solubility, and the presence of functional groups or heteroatoms. These properties are the basis for separation of the compounds and for specific detection signatures.

Online detection of chemicals in the environment generally follows the methodology outlined in Figure 7. The specific detection systems for online analytical chemistry will be described below with reference to this generic diagram. A raw sample stream of air or water is continuously pulled through sampling plumbing. The rate of flow of the sample stream is generally as large as practical in order to obtain a sample in a short period of time. A collector/concentrator is placed into this sample stream in order to gather a sample for presentation to the detection system. This

collector/concentrator can take on many different forms depending on the type of sample (air or water). This device may be an element that recognizes (and collects) a certain type of chemical or it may be a filter or sorption device for more generic collection.

The collected sample is then injected into a sample separation device that either temporally or spatially separates individual components. The process for introducing and separating the sample often uses the technique of Flow Injection Analysis (FIA). This technique uses a clean carrier fluid that continuously flushes the system. At a selectable point in time, a sample pulse is injected into this carrier stream. Hence the separation device can be nulled out before injection and return to baseline can be monitored. This technique allows for correction of drift in the separation and detection system performance. The time or space points of the separation can be used as part of the identification algorithm since generally the degree of separation depends on such molecular parameters as solubility, physical size, hydrophobicity, and dipole moment. However, the separation device does not generally have the stand-alone analytical capability for identification of a substance. Also of note here is that the sample collection, concentration and separation is usually the rate-limiting step in the analytical chain. The identification tools that are used for chemical compounds are generally quite rapid. Finally, the separation device often provides critical information regarding the quantification of the substances of interest.

At the outlet of the separation device, the essentially pure compounds are sequentially presented to the detection device for acquisition of molecular information. The specific types of detection devices applicable to this problem will be discussed below but the generally the tools use optical spectroscopy or Mass Spectrometry (MS) for the broadband detection of the compounds of interest. The information from the sample collector, separation device and detector is then presented to a data processing system containing an identification algorithm. This system rapidly identifies the compounds and provides quantification information to the reporting system that would typically be networked into other sensor systems and a central incident handling system.

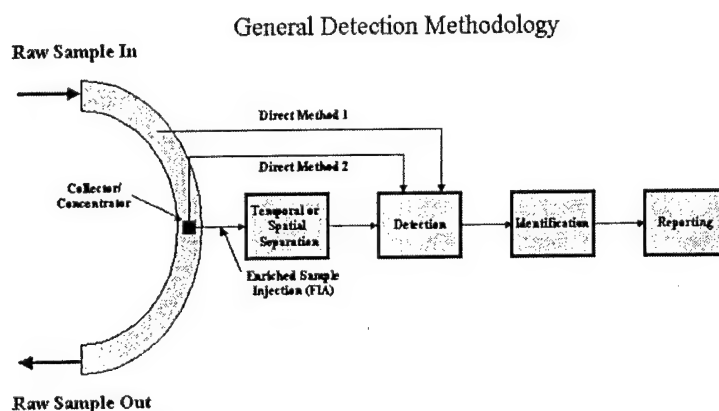


Figure 7. Generic Detection Methodology

III. Specific Detection Systems

The specific detection systems described next are currently reasonably well developed and are either fielded or nearly ready for fielding. The systems are described relative to the type of sample that they are equipped to handle. Several of the systems have been developed specifically for the detection of CWAs and are being modified to detect TICs and TIMs.

A. Vapor Phase Compounds in Air Samples

The detection of vapor phase compounds in air include some of the better developed technologies for agents with moderate vapor pressures. However gaseous compounds with very high vapor pressures present a significant analytical challenge. Furthermore, the speed of detection must be improved to provide the desired near-real-time detection.

1. Air Sampling Technologies

Sampling for compounds with moderate vapor pressure from air is generally achieved using either a cold surface or (more commonly) materials with high surface area and a propensity to adsorb organic compounds as opposed to water vapor (such as Tenax). In this application a volume of air is sampled through a column containing these materials. The organic compounds thus collect on the column and can be released into the separation device by heating the tube in a flow of carrier gas. Key technological developments needed for air sampling are materials with enhanced collection efficiency and lower pressure drops such that a larger sample could be acquired more rapidly. Several groups are working on this including commercial supplier of Tenax and a group at the Auburn University.

2. Separation Technologies

Once the sample is collected it is generally a complex mixture of many different chemicals. These are generally separated by GC. This process uses a carrier gas to pass the collected material through a specially coated column. Based on the differential solubility (strongly dependent on molecular properties) of the compounds on the column coating material, they separate out such that at the outlet of the column pulses of pure compounds can be sent to a detector. The times of arrival (under fixed conditions) are a property of the compound that can be used to help with identification. In standalone GC, generic detectors such as flame ionization devices are employed. These devices simply monitor the organic load exiting the column. However, detectors have been produced that are specific for halogens, sulfur or phosphorous. These types of devices produced by CMS Field Products Group have been used extensively for monitoring chemical weapons storage and destruction sites. They are extremely sensitive and accurate but as configured with these specific detectors they are not applicable to monitoring for TIC/TIM. Furthermore, the time to detect is several minutes as currently configured. However, several organizations are investigating "fast GC" options. Two leading organizations in this area are RVM Technologies and Louisiana State University. Also as described below, other detector devices can be readily interfaced to the GC.

3. Detection Technologies

a. Surface Acoustic Wave (SAW) Device

The SAW device is based on change in resonant frequency of a piezoelectric structure depending on the mass of material on the surface. This measurement can be made very rapidly and accurately, however a basic SAW is simply a device that measures mass on a surface, not very specific. In fact in this mode it is used as a detector for GC. However, specificity can be gained on the SAW by the application of a special polymer coating that preferentially adsorbs compounds of interest. A leading organization developing these materials is the NRL. In fact, this technology has been chosen as the basis for the Joint Chemical Agent Detector. The detection is reasonably rapid (< 1 min) but has been found to be somewhat dependent on atmospheric conditions. However recent developments have largely solved these problems. A key concern in the application for TIC/TIM detection is the development a broad range of polymers that allow detection of these materials.

b. Ion Mobility Spectrometry (IMS)

The IMS device is a time-of-flight device using a radioactive source or a glow discharge to place a charge on the vapor phase sample held at atmospheric pressure. Once the molecules in the sample are charged, they are pulsed into the IMS device where they separate in time based on their ion mobility that is related to the cluster size of the ion packet. This ion mobility is roughly related to the molecular mass of the material in the sample. This system is widely used for the detection of CWA and is the currently fielded system in the US and UK. The leading manufacturer of these devices is Graseby Ionics in the UK. The detection is quite rapid with the Time of Flights (TOFs) in the millisecond regime. However the resolution of the device is such that it would be difficult to use to measure a broad range of TIC/TIM compounds. However, it has successfully been integrated with a GC to produce much better results on a broad range of compounds. The device is quite sensitive and is a good candidate for this application.

c. Mass Spectrometry

The mass spectrometer is a vacuum based technique that also requires that a charge be placed on the sample of interest. However, since this is done at low pressure, no cluster formation takes place and the parameter that is measured is the true mass of the molecule. Furthermore in the ionization process, molecules are reproducibly fragmented such that a true molecular signatures are obtained. The mass spectrometer is the gold standard laboratory tool for identifying and quantifying molecular species. For instance the NIST library of mass spectral data includes $> 10^5$ compounds. The technique is extremely sensitive and specific when coupled to the gas chromatograph. The technology has always been hindered from going to the

field due to the large size, weight and power requirements. Also the GC has always been a device that requires a significant amount of time to do detection. As described above, the GC is now experiencing excellent progress toward high speed operation such that total time to detect can move into the time frame of < 1 min. This is a significant effort around the world, with leaders including Inficon (HAPSITE), the National Laboratories, Finnegan Inc., and the Johns Hopkins University.

d. Infrared Spectroscopy

Another tool that can be used for detection of compounds in air is infrared spectroscopy. This technique is based on the unique chemical bond stretching frequencies exhibited by organic molecules. These signatures are directly related to the chemical structure of the compound. This technique also has the advantage that it can be used in a non-contacting (remote) fashion. However, it suffers somewhat from low sensitivity and poor performance in a complex environment. It can also be coupled to a GC but the capability for standoff detection is removed when this is done. Infrared spectroscopy plays a very important role for very low molecular weight substances with very high vapor pressures. These compounds are difficult to capture on sorbent materials. Additionally, they are generally not the most toxic compounds such that a threat quantity would be quite high. This plays to the strength of the infrared technique in that it need not be supremely sensitive. It is extremely rapid, such that it could be a key component in an overall detection scheme discussed below.

4. Systems for Airborne Vapors

With reference back to Figure 5, a system of collectors, separators and detectors must be incorporated to provide the near real-time detection of vapor phase TIC/TIM in the environment. When the threat from TIC/TIM is analyzed, there is an extremely broad range of chemicals to be detected. Probably the most successful strategy for the near term is to take the gold standard laboratory tool, the GC-MS, and work to provide enhanced speed of operation and fieldability, while maintaining laboratory quality spectral performance. Such a system is currently under development at The Johns Hopkins University and parts of such a system has been developed and commercialized by Finnegan, Inc. The heart of the system is a high-resolution TOF mass spectrometer that measures at a rate of > 5000 samples per second. The TOF is fed by several different sources of vapor. In the normal operating mode, a sorbent material is used to sample environmental air at a repetition rate of one per minute. This material is thermally desorbed onto the column of a fast GC that runs at an equivalent repetition rate. In this mode, sample is concentrated and a full spectral scan (at a mass spectral resolution of > 2000) is acquired. The data is analyzed by algorithms that effectively prune the NIST database such that near-real-time detection is achieved. Since there is some dead time in the mass spectral analysis, direct mode inlets can also be sampled by the TOF. These inlets sample the air directly looking for the highly volatile compounds that are not captured on sorbent tubes. This system provides essentially full coverage of the TIC/TIM vapors in the air. However, at the current state of

development, it is not ready for fielding in a warfighter environment. However, in the near future the size, weight and power concerns could be readily addressed.

B. Particulates and Aerosols in Air Samples

Other key airborne threats are compounds with very low vapor pressures, typically presented as aerosols or particulates. These materials are extremely difficult to detect with current vapor sensing technology. Three detection approaches, involving Mass Spectrometry, and one using IMS seem to hold the most hope for these materials. A system that is currently fielded by the US Army is the Chemical Biological Mass Spectrometer (CBMS), which is now in the second phase of development. This system uses a frit to collect solid phase material. This frit is then heated up to vaporize the sample and the vapor is pulled into an ion trap mass spectrometer where electron impact ionization signatures can be obtained. The second approach is also being developed by the US Army and the British. It is based on collecting particulate material into a liquid. This material is then run through a liquid chromatograph for separation and then into an electrospray ionization mass spectrometer for analysis. This system should provide superior information for the larger TIC/TIM compounds because it uses a softer ionization technique that provides enhanced molecular identification. This system is at an intermediate stage of development. The concepts have been proven out for larger molecules such as toxins and prototyping has begun. A third option, based on Matrix Assisted Laser Desorption/Ionization (MALDI) Mass Spectrometry, is being developed at The Johns Hopkins University. It uses an aerosol impactor to collect sample, in the solid phase, onto a polymer tape. This sample is processed and then moved into a small time-of-flight mass spectrometer for MALDI analysis. This method is also a softer ionization tool and provides better molecular signatures. This system is also at an intermediate stage of development. It has been field tested and has had extensive set of tests run in the laboratory. At this stage however, it is too large for this sort of deployment. The final system that potentially addresses these low vapor pressure compounds is being developed at the US Army also. It is based on collection of a sample onto a frit (as in the CBMS) where it is vaporized and passes through a short GC column (to provide some separation capability) and then onto an IMS device. This system has been tested several times at field trials and has performed well for biological agent simulants. It may perform against the TIC/TIM materials if it has the proper analytical capability.

C. Chemicals in Water Samples

The detection of chemical compounds in water is a problem that has been addressed over a long period of time by the USEPA. Most of the methods are based on the gold standard assay, GC-MS. Significant numbers of analytical methods have been developed but many of them are discrete, not online, assays. Water samples are collected and then extracted with various methodologies to concentrate the sample of interest and minimize the water background. These methods are not particularly amenable to the problem at hand, but do provide great standards to shoot for. Several online methods have been developed around Membrane Inlet Mass Spectrometry. In this technique a silicone rubber membrane separates the liquid sample from the vacuum of the mass spectrometer. Certain classes of compounds traverse the membrane and enter the mass spectrometer whereas almost none of the water comes across the membrane. This technique has proven very sensitive for non-polar and halogenated organic compounds. It suffers, however, from not being

coupled to a separation technique. All of the compounds present in the water sample that can traverse the membrane, do so, causing a cluttered mass spectral result that is difficult, if not impossible to deconvolute. Recent work at University of Hamburg and University of South Florida has centered on using the membrane to sample the material from the water where it is passed into a GC effluent for separation. This approach holds great promise for that class of TIC/TIM compounds that will cross the silicone rubber membrane. Another technology of great promise for the field and is quite common in the laboratory is solid phase extraction. In this technique, other types of polymers can be used as sampling substrates to adsorb organic compounds from water. A variety of polymers can be used to sample many different types of compounds in water. After the sample is collected, it is thermally desorbed into a GC-MS for analysis. Current systems for running multiple samples use robotic sampling and injection. This is a somewhat clumsy apparatus for field operation. It seems perfectly reasonable, however, to produce a system with a multiple polymer assembly in a tape format that could be readily implemented in an online fashion. I do not know of work currently being conducted on this but it seems feasible. Finally, an option for certain types of organic compounds in water is Raman spectroscopy. This spectroscopic technique is similar to infrared in that it measures vibrational features of the molecules of interest to produce a fingerprint. Unlike Infrared however it can be used on aqueous samples because the water resonance is not excited by Raman spectroscopy. Conventional Raman spectroscopy is a low sensitivity technique. However relatively recent work on surface enhanced or resonant Raman spectroscopy has increased sensitivity by many orders of magnitude. It is feasible to develop specialized sampling substrates that could be used to collect samples and provide the proper surface for the resonant Raman effect. This may be an excellent complement to the GC-MS systems particularly since significant effort has resulted in readily portable Raman spectrometers.

IV. Future Trends

The discussion thus far has focused on the prospects for solutions in the reasonably near future (<5 years). There are significant developments underway to move to the next generation of analytical instruments that may be amenable to online detection work. A large effort has been carried out at the National Laboratories (primarily Oak Ridge and Sandia) on using the new tools of Micro-Electromechanical Systems coupled with advanced photonics to miniaturize analytical equipment. This includes micro chromatography such as GC, liquid chromatography and capillary electrophoresis. In particular, researchers at Sandia are actively working to produce an analytical chemistry laboratory on a chip. This system would include sampling systems, chromatographic separations, and detectors. This work is quite promising but will not be ready in the near term. In separate projects at Oak Ridge and at Northrop Gruman work has been carried out on miniaturizing different versions of a mass spectrometer. The systems produced to date do not have the analytical capability of a laboratory instrument so would not make a good near term choice but over the next 10 years the situation could be drastically different. It seems clear that there are great advances being made daily on these technologies. A reasonably portable system can be put together using near term emerging technology as the basis that will provide a great deal of protection to the warfighters in the field.

Chemical-Based Biosensors by Dr. Amanda Jenkins, USARL

Summary

Chemical biosensors are sensors that combine advanced chemical materials such as dendrimers and imprinted polymers and biological materials such as antibodies and enzymes. This combination takes advantage of the strengths of chemistry and biology to overcome the limitations of each type of science. This allows the sensors to provide real-time detection and classification of live and dead biological species, as well as for determining the presence of TICs of interest, particularly at low levels. The sensors generally consist of a micro-concentrator pump, capture probes with signal transducers, and a fiber optic detection platform with data collection electronics and software. The technology for sensitive detection of a variety of analytes is expanding rapidly. However most of this technology lacks the ability to provide specific detection of one analyte versus another with no false positives. One of the most common approaches to combat this lack of specificity is the use of biological recognition materials such as cells and living creatures. These techniques are limited by the critical environmental conditions that must be maintained for healthy organisms. An alternate approach is to use chemistry and synthetic materials to simulate the abilities of these biological species while decreasing the environmental restraints that need to be maintained in order to keep them functional. Using such chemical techniques we are able to create materials that can be used with commercially available off-the-shelf technologies to create chemical biosensors.

Chemical biosensor systems provide an unprecedented configuration that involves a number of nanomaterials-based technologies that include nanocapsule designs and syntheses, dye/protein encapsulation and immobilization, pre-concentration of oily chemical agents in water, preparation of molecularly imprinted polymer, MIP-based receptors, lanthanide metal-based real-time target recognition, and optical signal transduction mechanisms. This is the first time that materials based sensors have ever been successfully demonstrated for the real-time activity detection and identification of chemical and biological agents. The key for this success is the incorporation of newly developed nanofunctional and nanobiomimetic materials. These materials are not only able to mimic antibody recognition functions, but also transduce the signal in real-time without the need for fluorescent tags. Furthermore, the proposed nanomaterials can be engineered to perform trigger detection directly or to carry a variety of active ingredients (i.e. dyes or enzymes) to achieve activity detection indirectly. The Limit of Detection will at low ppt levels for chemical agents, which is at least three orders of magnitude better than those obtained from the best optical sensors to date. The resulting nanodevice can be used as a stand-alone trigger and identifier in TIC point detection (i.e. air and water monitor) applications. This technology will revolutionize current sensor designs in terms of size, weight, performance, and logistics.

A Brief Overview of the Existing Detection Methodologies

With increased concern over potential hazardous effects of TIC materials in the air and water in recent years, a variety of detection technologies have been tested. These technologies range from traditional instruments (i.e. GC/High Pressure Liquid Chromatography, fluorescence, MS, Raman, and Fourier Transform Infrared (FTIR)) to newly developed immuno/PCR/enzyme/materials based assays and sensors (i.e. electrical, and optical/laser based sensors). While mainly designed for the purpose of structure-based identification, none of them are capable of detecting the activity or function of the biological species or systems in situ without chemical separations or sample preparations. This is primarily due to the fact that these systems possess significant interference problems, and are only capable of detecting analytes at levels that are orders of magnitude higher than those required for bio-

activity detection. In addition, the size, weight, and logistics of the instrument-based detectors prohibit their use in the field, whereas the lightweight bioassays and sensors are generally target specific, and in many cases, require multiple steps for sample preparation. Broad-spectrum detection and classification are primarily achieved using spectroscopic techniques, cell/tissue-based sensors, or novel materials. Spectroscopic techniques (i.e. Raman and FTIR) are generally insensitive, non-specific, and prone to a variety of interferents. With the help of nanopatterned materials, sensitivity may be enhanced significantly (i.e. Surface Enhanced Raman Spectroscopy), but it is still far away from the detection level required for biological activity detection. On the other hand, cell and tissue-based sensors show great potentials for classifying as well as identifying both existing and new chemical and biological toxins in the environment. Data collection with these sensors is based on the direct measurement of cell or tissue responses upon exposure to toxic agents. Although the sensors exhibit superior selectivity towards a variety of agents, they suffer serious stability problems, and a change in temperature, oxygen content, buffer or aqueous media could cause a malfunction.

Recently, a variety of synthetic materials have been incorporated into chemical and biological agent detection systems. While most of them serve as tagging molecules (i.e. quantum dots and up-converting phosphorescent particles) or linkers for the attachment of receptors (polyethylene glycols or dendrimers), few function as both capture probes and signal transducers. Conductive polymers are the only commercially available polymers that are currently capable of performing class detection as well as signal transduction for some chemical agents. However, these sensors are generally insensitive (only detect ppm levels of agents) and prone to a variety of interference problems including temperature and humidity.

Nanocapsule/Dye-Based Detection

Biological activities have been detected using organic dyes embedded in dendritic polymer based nanocapsules. Dendritic polymers generally include dendrimers, dendrigrafts, and hyper-branched polymers. Since dendrigrafts are often constructed with larger molecular building blocks such as oligomers and polymers instead of monomers, they tend to be much larger in size and less dense in the interior when compared with dendrimers. This makes their nanocontainer properties even more attractive than that of dendrimers, since the cargo space of the latter is very limited. Due to the fact that hyper-branched polymers derived from convergent self-condensing polymerization are structural analogs of PEOX dendrigrafts, we first utilized PEOX dendrigrafts as model polymers for many of the encapsulation and immobilization related structure-property studies. However, for large-scale production, low cost hyper-branched PEOX polymers with similar properties will replace the corresponding PEOX dendrigrafts. These dendrigraft polymers are soluble in both water and a variety of polar organic solvents. The facile design allows the preparation of a variety of nanostructured materials for the immobilization of different organic dyes on solid substrates. In addition, these novel materials with different functional groups are capable of capturing different classes of bio-species based on their surface charges, hydrophobicity, and receptors present at the surface of the biomolecules or cells. For example, sialic acid can be incorporated into the polymer matrix to capture viruses. Others can also be prepared for the generic capture of spores, bacteria, and toxins.

Functional polymers can also be utilized for the encapsulation/immobilization of organic dyes and for enhancing their detection capabilities. A number of candidate polymers have been prepared and tested in both solution and thin films for live bacteria detection. Preliminary data indicates that hydrophobe modified polymers tend to give lower fluorescent signals due to the fact that the hydrophobic surface groups can serve as a stronger barrier layer to prevent organic dye molecules (also hydrophobic) from penetrating into the cells. Moreover, the positively charged polymers prohibit the dye molecules (also

carry positive charges) from being encapsulated/immobilizing into the polymer matrix prior to mixing with bacteria, thus generating lower fluorescent signals. The best polymer based immobilization systems are the carboxylate-modified nanocapsules, in which the surface charges can be modulated based on pH changes (similar to the cell membrane). Such nanocapsules can weakly associate with positively charged organic dye molecules, and upon mixing with live cells, give significant signal enhancement. For example, when SYTO9 was first mixed with a carboxylate modified nanocapsule and then used for the detection of live *Erwinia Herbicola* (bacteria agent simulant), a dramatic signal enhancement of about 4 fold was noticed when compared to the sample without the nanocapsule. Other polymers, with the same size, shape, and chemical composition but different surface groups showed limited fluorescent signal enhancement. SYTO9 and propidium iodide mixtures were also incorporated into the above nanocapsules, and it was found that the detection sensitivity for live *Erwinia Herbicola* was also enhanced. The presence of live versus dead bacteria can be detected based on this dye/polymer composition (Figure 8). This discovery shows promise for the detection of live biological species from a complex biological mixture. The proposed polymeric system is advantageous over commercially available polyamidoamine dendrimers, whose sizes and interior space can not be altered readily. In addition, the proposed polymers can be attached to a surface through covalent linkages allowing a microarray based sensor to be prepared. Other dyes could also be incorporated into these systems through a simple nanoencapsulation step. Using different dyes will all this technology to be adapted for the detection of toxic industrial chemicals and to determine the effects of these chemicals on living systems.

Figure 8. The addition of the carboxylate modified nanocapsule also enhances live bacteria detection in the presence of a biological mixture (top). The lower intensity of the live bacteria region (middle) is due to the lower concentration of SYTO 9 (less than 50%) utilized in the mix dye formulation. The bottom line represents the signal generated from Propidium iodide with dead *Erwinia Herbicola*.

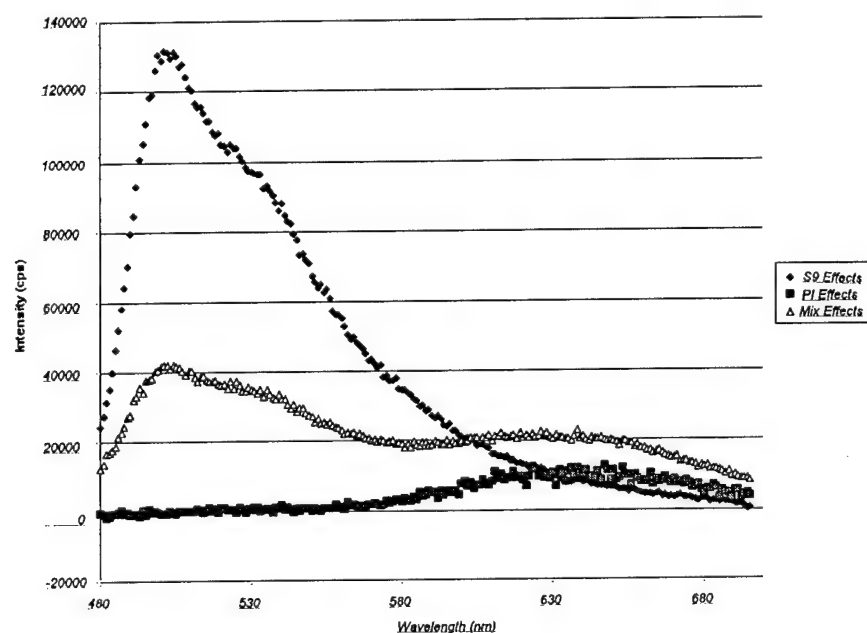


Figure 8. Dye/Polymer Composition

Chemical Detection

Natural waters are contaminated with various pesticides and insecticides because of their widespread use in commercial and residential applications. These chemicals directly applied to the ground are rapidly transported into the groundwater. In 1988, over 46 pesticides were determined in the ground waters of the US. Despite today's technology, detection of environmental pollutants like pesticides, insecticides, and herbicides at the levels specified by the USEPA remains a challenge. Many pesticides and insecticides produce the same type of cholinesterase inhibition in the body as nerve agents but at lower levels. The side effects of extended exposure include rashes, fatigue, muscle and joint pain, headaches, loss of memory, depression, abdominal pain and diarrhea, coughing, sneezing, choking sensations, chest pain, sleep disturbance, and hair loss. Many of these materials are also suspected carcinogens.

Researchers at the USARL have prepared novel polymeric materials containing artificial recognition sites for pesticide and insecticide detection in water. The materials are designed to selectively detect phosphonate-containing species such as pesticides, insecticides and herbicides. They are based on molecular imprinting techniques combined with sensitized lanthanide luminescence and function by selectively binding the phosphonate group to a functionality-imprinted polymer possessing a bound luminescent lanthanide ion. Polymers have been imprinted for Pinacolyl Methyl Phosphonate (PMP) (the hydrolysis product of the nerve agent sarin) and over a dozen organophosphate pesticides. The polymers are coated onto multi-mode optical fibers, and evaluated by luminescence between 550-700nm with a miniaturized fiber-optic spectrometer. The luminescence signatures change when the analyte is reversibly bound to the lanthanide in the co-polymer. The polymers respond to increasing concentrations of analyte with an increase in luminescence intensity. The resulting peak areas in the 609 to 621 nm spectral region are calculated and plotted as a function of concentration. The limits of detection for these sensors are in the low ppt with linear ranges from low ppt to ppm. The response time of the polymer shows a positive response to the presence of analyte after 3 min for pH values from 6-12 and a positive response after 1 min for the solution with a pH above 10. Neutral and slightly basic values (pH from 6-11) provide a steeper, more linear response that is consistent over the entire pH range. The polymers exhibit the same recognition characteristics over several months of use.

Many pesticides and insecticides are chemically analogous. Common pesticides, insecticides and herbicides were tested against the sensors in order to determine the degree of interference. The concentration used for screening interferences, 100 ppm, is much higher than typically found in water systems even with runoff from nearby agriculture. None of the pesticides tested against the PMP hydrolysis product sensor responded as interferents. The influence of these chemicals was apparent as indicated by the changing intensity of the major 615 nm europium luminescence band, however none produced a luminescence peak in the 610 nm region. Since the chemicals that are the most likely interferences do not cause false positive readings, other less similar compounds should be unlikely to interfere. In addition, none of the pesticides screened irreversibly bound to the sensors so poisoning is not a concern. The polymer imprinted for chlorpyrifos methyl, a small pyridine organothiophosphate pesticide not only discriminates against other classes of pesticides but also can distinguish against other larger members of its own class such as chlorpyrifos ethyl. The spectra resulting from the exposure of selected pesticides at 100 ppm with that of 1 ppm chlorpyrifos methyl is shown in Figure 9. This type of investigation is particularly important for those individuals who do not show adverse reactions upon exposure to low-levels of organo phosphanates, but will develop chronic illnesses in later years.

Molecularly imprinted polymer technology has also been used to develop a variety of materials for the selective sequestering of many TICs including heavy metals such as lead and uranium. If a signal

transduction mechanism can be incorporated into these polymers in the way the lanthanides are used for the detection of the pesticides and insecticides selective detection and quantification of TICs including organic solvents and heavy metals can be realized.

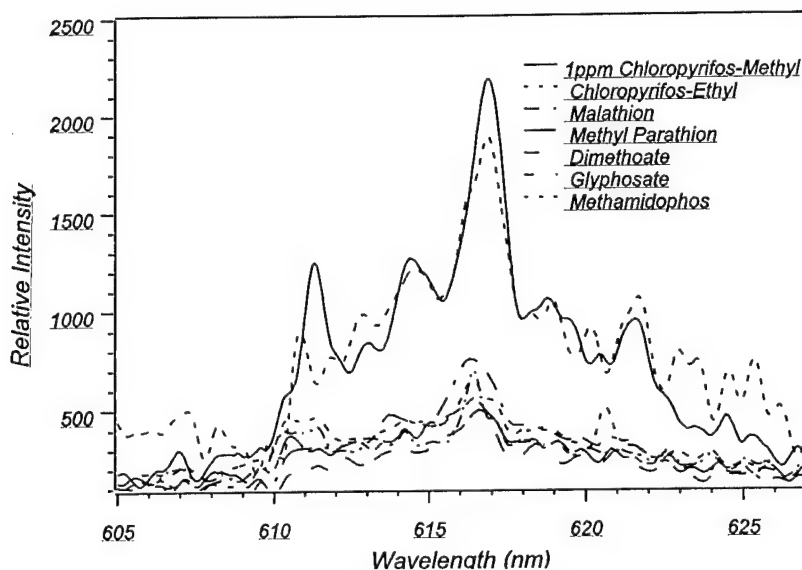


Figure 9. Response of The Chloropyrifos-Methyl Sensor to 100 ppm Interferences

Micro-Pump and Sample Concentrator

The proposed nanobio and nanofunctional materials based sensor system can be further integrated into a microarray platform and be coupled with the micro-pump based sample concentrator (Figure 10). The capture complexes will be excited using a series of Light Emitting Diodes at various wavelengths and the emission luminescence will be monitored with a miniaturized fiber-optic spectrometer. The sample concentration system consists of a miniature pump, a fluid distribution and filtration chamber and the sensor elements. A key element of the system is the micro pumping unit that continuously draws samples from the environment. Another element in the system is the fluid distribution chamber where, different filters can be used to pre-screen the samples according to the particle size. A rotating fluid regulator will be fabricated to send filtered fluid to different sensor elements, which are arranged like the airport boarding docks. Unlike existing sample concentration systems, the present sensor element can also be integrated with the collecting material for sample concentration. Utilizing the sensor system, the concentrations of a variety of chemicals can be more accurately detected, and the exposures to different toxic materials can be rapidly screened, identified, and treated without the need for tedious laboratory protocols and use of trained personnel. Novel sensor system designs such as this will greatly enhance the existing operational capabilities for TIC point detection and to assist in medical diagnostics.

Technical Challenges

Determination of low-level exposures to toxins in the environment particularly in mixed systems is challenging. Potential exposure to a complex mixture of toxic materials and their decomposition products in the environment makes medical diagnosis and assessment of exposure consequences even more difficult. Exposure effects to unknown threats above and below LD50 are almost impossible to

predict with existing technology. Therefore, real-time signal transduction and ultra high sensitivity detection are absolute requirements for detecting these materials in the environment. These requirements have raised a tremendous technical barrier for scientists from different disciplinary areas

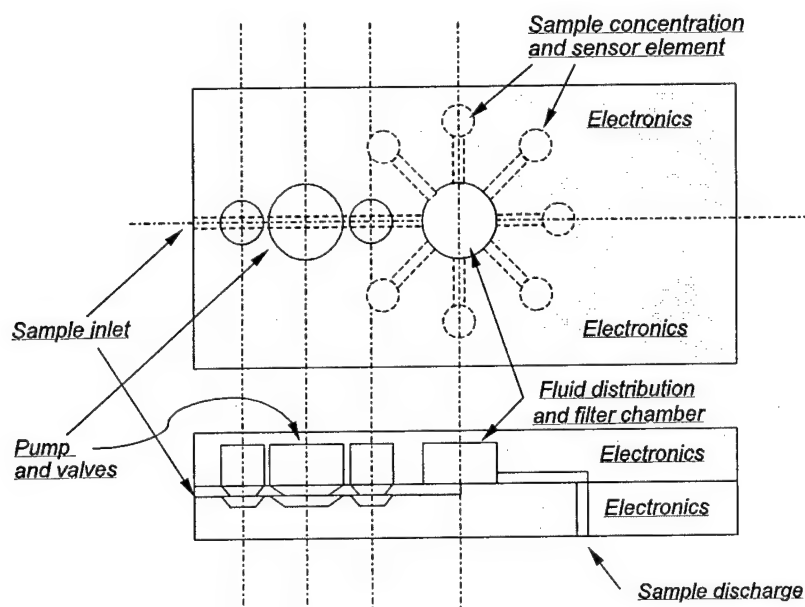


Figure 10. Illustration of an Integrated Chemical and Biological Agent Detection System

including chemistry, biology, medicine, physics, device engineering (electronic, optical, and mechanical), as well as data mining and processing. To address these issues, a variety of nanofunctional and nanobiomimetic materials can be synthesized as capture probes for TIC detection and low-level exposure assessment. The proposed capture probes will be capable of detecting as well as classifying and quantifying toxic chemicals in real-time at ppt levels. A micro-pump based sample concentrator will also be incorporated into the sensor platform allowing further sensitivity enhancement. The results generated from the proposed sensor system can be correlated with those obtained from the traditional laboratory based techniques.

Near-Term Achievements

Within the next five years this technology should be able to provide a real-time (less than 15 min) system for the detection of some TICs such as pesticides, insecticides, organic solvents, and selected heavy metals in the water. It should be battery powered and able to be carried by one individual.

Long-Term Achievements

In the long term, this technology should lead to a device capable of detecting a wide range of TICs in both air and water. The device would be adaptable and capable of detecting even previously unidentified materials of concern and complex mixtures. The eventual device would be handheld, and operated under low battery power and able to withstand exceedingly harsh conditions such as those found in "superfund" sites.

DNA-Based Microarrays by Dr. Joanne Andreadis, US NRL

This technical summary will discuss the impact of DNA-based microarray detection as an enabling near term technology (5-6 yr) for ESB systems designed for real-time, continuous exposure and health effects monitoring of TICs and TIMs, respectively. Recent technological advancements in microfabrication combined with the rapid acquisition of full genome sequence data have led to the development of DNA arrays. DNA microarrays are an indispensable platform that can provide high throughput processing and analysis of large amounts of information in a massively parallel manner. DNA microarrays utilize immobilized pieces of DNA (ranging in length from 25 to 1200 nucleotides depending on array specifications) concentrated onto specific areas of a solid support such as a specially coated glass slide or nylon membrane (Schena, 1999). Fluorescently tagged DNA, obtained from a sample, is then applied to the surface of the microarray under conditions that support specific hybridization, or binding, to complementary sequences immobilized on the surface of the array. The presence of a fluorescent signal at a defined location on the microarray enables both identification and relative quantitation of DNA/Ribonucleic (RNA) species in the sample. DNA microarray technology will significantly impact TIC/TIM by 1) providing high throughput analysis capabilities to supplement the development of multiple sensor systems including enzyme, antibody, and nucleic acid based detection platforms and 2) expanding current tissue and/or cell-based sensor potential. Additionally, DNA microarray-based analysis is an enabling technology that can be used for diagnostic evaluation of clinical samples and high throughput screening of food and water supplies for direct detection of nucleic acid sequences indicative of bacterial or viral contamination. Chumakov and co-workers have pioneered the development of oligonucleotide arrays that identify and discriminate between several foodborne human pathogens (Chizhikov et al. 2001). The use of microarrays for this application is highly promising as this technology is conducive to multiplex analysis and automation. As these topics are beyond the scope of the white paper, this summary will focus on DNA array technology and its impact on tissue and cell-based sensor platforms.

Conventional methods for the detection of environmental threats are primarily based on chemical, antibody, or nucleic acid based assays that rely on molecular recognition to identify a particular threat or agent. Whereas these methods are effective for the identification and quantitation of specific molecules, they are generally low-throughput and limited in their ability to 1) detect unknown or unanticipated threats, 2) detect combinatorial threats or synergistic effects of TICs and TIMs, and 3) predict human performance consequences caused by low-level toxicity or chronic exposure. Unlike immuno- or nucleic acid-based assays, cell-based sensors provide general and physiologically relevant information about a wide variety of substances. Cells are optimal biological elements for generic detection as they contain a variety of enzymes, receptors, channels and pathways that enable them to respond dynamically to their environment and have been utilized for a variety of applications including routine toxicological analysis and assessment of environmental contamination (Applegate, 1998; Belkin 1998; Burlage 1994). Cell-based sensors fill an important threat assessment niche as they are designed to simultaneously detect large numbers of both known and unknown threats, characterize the functionality of those threats, and to some extent predict human performance upon exposure (Stenger et al., 2001, Pancrazio et al., 1999). Currently, tissue slices and cultured biological cells are used as biological elements in devices that utilize either extracellular recordings or optical measurements to evaluate cellular response in cell-based sensor platforms (Gross et al., 1992, Gross et al., 1995, Pancrazio et al., 1999). This approach, however, may be limited in the range of threats that can be detected because of the necessity to monitor defined cellular pathways or need to utilize specialized cell types such as electrically active neurons or cardiomyocytes.

The advent of functional genomics, however, provides an exciting new avenue for toxicological assessment via cell-based sensor detection that is based on the transient induction of gene modulation events. The potential of functional genomics to identify unique gene expression patterns or "signatures" indicative of toxic exposure in a variety of cell types (such as neurons, epithelial cells, and immune cells) would expand the detection capabilities of current cell-based sensor platforms. Specifically, one can envision a three-part automated flow through system in which (1) cultured cells, grown in two or three dimensional matrices, are exposed to media infused with concentrated air or water samples, (2) the cells are then washed and utilized directly for enzymatic amplification and labeling, and (3) cell response profiles obtained through the analysis of disposable microarray cartridges. Identification of an unknown compound or class of compounds may be obtained through the comparison of observed gene modulation in a particular cell type to a library of cell response profiles. Mirzabekov and co-workers, have developed a similar system for the direct detection and discrimination of pathogenic sequences from *Escherichia coli*, *Bacillus subtilis*, and *Bacillus thuringiensis* (Bavykin et al. 2001).

The laboratories of J. Jett, D. Relman, and co-workers have utilized DNA microarrays to identify gene modulation events in circulating peripheral mononuclear cells and other tissues indicative of toxic exposure. Although much of this work has focused on host cell response to infectious agents, the work suggests that exposure to toxic levels of chemicals or chemical mixtures, such a high levels of JP8 fuel, may be determined from the analysis of gene modulation in cells obtained from a blood sample. Recent work in our laboratory has utilized DNA microarrays to examine whether cultured neurons, grown on a two dimensional surface, could be used to obtain reproducible gene expression profiles that would indicate exposure to a class of toxicants. Using this approach, reproducible and distinct temporal profiles of gene expression were obtained from cultured neurons following exposure to mechanistically related chemicals such as trimethylolpropane phosphate (TMPP) and bicuculline (Andreadis et al., 2001). Our findings, and that of other research groups, suggest that temporal gene expression profiles analyzed by clustering algorithms could be utilized to identify probable pathways of gene expression involved in specific physiological responses and enable overall comparison of profiles that might distinguish toxicants. Larger experimental sets, involving cell exposure to different families of toxicants must be generated to establish a library of gene expression profiles that predict and/or classify known and unknown threats.

Microarray-based detection is appropriate for near real time detection (defined in terms of minutes to hours). A typical microarray experiment from cellular exposure to analysis could be performed within hours using current technology. A model experimental time-table would include the following parameters: 1) minimal 25 min post cell exposure for RNA transcription and translocation to the cytoplasm, 2) approximately 30-45 min for sample preparation (although purification methods are being developed by commercial vendors to accelerate this process), and 3) a minimum of 30 and maximum of 16 hr for hybridization and analysis. DNA microarrays can detect approximately 50 copies of a DNA/RNA species present in a sample; however, protocol optimization should enable an improvement in sensitivity that would approach 10-20 copies. Potential advantages for military field applications that utilize DNA microarray technology is high-throughput detection, multiplex interrogation for reliable threat assessment, compatibility with use of lyophilized reagents, compatibility with automation, microarray shelf-life of 6 months to a year, application of a single assay for a variety of cell types, and low production cost post development. Challenges of this approach primarily involve the cellular component of the sensor including climate effects and ability to withstand rough handling. Climate-related issues may be circumvented through the use of an automated environmental control system that could be used both to maintain the cellular component of the sensor and to optimize conditions for hybridization.

Important technological advances in high throughput microarray detection have taken place over the last 4 yr that enable tens of thousands of DNA sequences to be accurately immobilized on nylon and glass supports. The realization of a portable sensor capable of employing functional genomics, however, will require the miniaturization of robotic systems currently available for automated sample processing. Although DNA microarray technology appears promising, targeted research efforts in the short term must address important challenges regarding the development of reliable surface chemistry to enhance shelf-life and robust signaling, sample preparation including isolation and labeling strategies, evaluation of reproducibility (determination of required replicates, anticipated variation, effect of field conditions), and lastly the development microfluidics and automation capabilities. The timeline for development of this technology would be dependent on man-hours of dedicated research effort and funding availability. Several laboratories in the public and private sector are exploring both attachment chemistries and novel microarray surfaces to enhance hybridization between soluble labeled sample and immobilized DNA. Sample isolation/preparation is also an active area of research particularly in the private sector because of the applicability of any developments to a variety of lucrative commercial markets. Several methods recently developed utilize sonication or novel aspects of nucleic acid binding chemistry. This is clearly an important area that needs further development for a variety of detection platforms. The development of alternative sample labeling approaches that could replace the commonly used cyanine dyes would greatly improve the applicability of microarray-based technology to laboratory and field applications. Specifically, our laboratory and others are investigating the use of luminescent nanocrystals (quantum dots) and plasmon resonance particles as multicolor, reporters with tunable low cross-talk emission. Potential benefits of detection platforms using either alternative labeling strategy are as follows: 1) potential simple excitation (no laser requirement) 2) single wavelength excitation with simultaneous multiple wavelength emission for multiple sample analysis in parallel, 3) narrow non-overlapping emission bands which will eliminate cross-talk between sample output, 4) resistance to photobleaching, 5) the potential for single molecule detection. Other important considerations for the use of microarray-based methods to assess host cell response include the type of cell culture system (tissue, immortalized cell lines, primary cultures), duration of exposure, sensitivity, and reproducibility. These are critical task areas that require further evaluation. Once the approach is validated, miniaturization and automation of the system can be explored.

More recent scientific advances have expanded the concept of microarrays to include systems that utilize bead-based systems (e.g. Luminex- FlowMetrix, Illumina bead arrays) and systems that utilize electrochemical signals (e.g. Motorola eSensor, Xanthon) rather than fluorescent or luminescent signals for nucleic acid detection. Other systems such as the Bead Array Counter developed by Coulter and co-workers, use magnetoresistance technology to detect DNA-DNA binding events (Baselt et al., 1998). Many of these systems are currently being evaluated and may be more appropriate for field-based assays. For example, certain bead-based systems enable the sample and array component to be mixed while both are in suspension, possibly resulting in higher sensitivity. Also, bead-based microarrays systems may provide a highly flexible format that allows instant customization of an assay based on bead selection.

The utility of microarrays as a toxicological assessment tool for field applications is promising and requires further exploration. The realization of a portable sensor capable of simultaneously employing functional genomics and perhaps even proteomics analysis techniques will require a concerted research effort. In summary, microarray-based technology has the potential to produce a highly sensitive toxicological assessment tool for screening air and water samples that could provide both physiologically relevant information regarding adverse health affects and predictive performance information for field personnel.

References:

- Andreadis J.D. et al. 2001. Identification of differential gene expression profiles in rat cortical cells exposed to the neuroactive agents TMPP and bicuculline. *Biosens. and Bioelectron.* In press.
- Applegate, B.M, et al. 1998. A chromosomally-based *tdi-luxCDABE* whole cell reporter for benzene, toluene, ethylbenzene, and xylene (BTEX) sensing. *Appl. Environ. Microbiol.* 64:2730.
- Baselt D.R., et al. 1998. A biosensor based on magnetoresistance technology. *Biosensors and Bioelectronics.* 13:731.
- Bavykin, S.G et al. 2001. Portable system for microbial sample preparation and oligonucleotide microarray analysis. *Applied and Environ. Microbiol.* 67: 922.
- Belkin, S. et al. 1998. A panel of stress-responsive luminous bacteria for monitoring wastewater toxicity. *Methods Mol. Biol.* 102: 247
- Burlage, R.S. et al. 1994. Bioluminescent reporter bacteria detect contaminants in soil samples. *Appl. Microbiol. Biotechnol.* 45: 731.
- Chizhikov, V., et al. 2001. Microarray analysis of microbial virulence factors. *Appl. Environ. Microbiol.* 67:3258.
- Gross, G.W. et al. 1995. The use of neuronal networks on multielectrode arrays as biosensors. *Biosens. Bioelectron.* 10:553.
- Gross, G.W. et al. 1997. Odor, drug, and toxin analysis with neuronal networks, *in vitro*; extracellular array recording of network responses. *Biosens. Bioelectron.* 12: 373.
- Pancrazio J.J. et al. 1999. Development and application of cell-based biosensors. *Ann. Biomed. Engin.* 27: 697.
- Schena, M, et al. 1998. Microarrays: biotechnology's discovery platform for functional genomics. *TIB Tech.* 16: 301.
- Stenger, D.A., et al. 2001. Detection of physiologically active compounds using cell-based biosensors. *Trends in Biotech.* 19: 304.

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LIST OF ACRONYMS

APD	Advanced Point Detector
BEWS	Biological Early Warning System
BWA	Biological Warfare Agent
CBMS	Chemical Biological Mas Spectrometer
CONUS	Continental United States
CWA	Chemical Warfare Agent
DDT	Dichlorodiphenyltrichlorethane
DNA	Deoxyribonucleic Acid
DoD	Department of Defense
ESB	Environmental Sentinel Biomonitor
FHP	Force Health Protection
FIA	Flow Injection Analysis
FTIR	Fourier Transform Infrared
GC	Gas Chromatography
IBAD	Integrated Biological Agent Detector
ICAM	Improved Chemical Agent Monitor
IMS	Ion Mobility Spectrometry
ITF	International Task Force
JFOC	Joint Future Operational Capabilities
JORD	Joint Operational Requirement Document
JSAWM	Joint Service Agent Water Monitor
LIDAR	Light Detection and Ranging
MALDI	Matrix-Assisted Laser Desorption/Ionization
MNS	Mission Need Statement
MS	Mass Spectrometry
NBC	Nuclear, Biological, Chemical
NIST	National Institute for Standards and Technology
NEPU	Navy Environmental & Preventive Medicine Unit
NRL	National Research Laboratory
OCONUS	Outside the Continental United States
OEH	Occupational and Environmental Health
ORM	Operational Risk Management
PAMAM	Polyamidoamine
PCR	Polymerase Chain Reaction
PM	Preventive Medicine
PMP	Pinacolyl Methyl Phosphanate
PRD	Presidential Review Directive
QSTAG	Quadripartite Armies Standardization Agreement
R&D	Research & Development
RNA	Ribonucleic Acid
SASO	Sustainment and Support Operations
SAW	Surface Acoustic Wave
STANAG	Standard NATO Agreement
TAML	Theater Area Medical Laboratory
TB Med	Technical Bulletin Medical

LIST OF ACRONYMS - CONT'D

TDS	Total Dissolved Solids
TG	Technical Guide
TIC	Toxic Industrial Chemicals
TIM	Toxic Industrial Materials
TMPP	Trimethylolpropane Phosphate
TOF	Time of Flight
UK	United Kingdom
US	United States
USACEHR	US Army Center for Environmental Health Research
USACHPPM	US Army Center for Health Promotion and Preventive Medicine
USEPA	US Environmental Protection Agency
USARL	US Army Research Laboratory
WET	Whole Effluent Toxicity
WQAS-ENG	Water Quality Analysis Set - Engineer
WQAS-P	Water Quality Analysis Set - Purification
WQAS-PM	Water Quality Analysis Set - Preventive Medicine

LIST OF SYMBOLS AND UNITS

C	centigrade
e	2.718...
hr	hour(s)
L	Liter
LC _{t50}	concentration and exposure time product lethal to 50 percent of those exposed
mg	milligram
mm	millimeter
nm	nanometer
pH	hydrogen-ion concentration
ppm	parts per million
ppt	parts per trillion
μg	microgram
yr	year(s)

PRIORITY RANKING LIST OF ITF-25 CHEMICALS FROM
DRAFT MNS FOR HAZARDS FROM INDUSTRIAL CHEMICALS

<u>Rank</u>	<u>Name</u>	<u>ITF-25 Rating</u>
1	Carbon disulfide	High
2	Ammonia	High
3	Ethylene oxide	High
4	Formaldehyde	High
5	Nitric acid, fuming	High
6	Diborane	High
7	Sulfuric acid	High
8	Chlorine	High
9	Boron trichloride	High
10	Boron trifluoride	High
11	Arsine	High
12	Hydrogen bromide	High
13	Hydrogen chloride	High
14	Hydrogen cyanide	High
15	Hydrogen fluoride	High
16	Hydrogen sulfide	High
17	Phosgene	High
18	Phosphorus trichloride	High
19	Sulfur dioxide	High
20	Fluorine	High
21	Tungsten hexafluoride	High